



Plant Xenogenic Expression

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Abstract

The objective of this report is to bring to light the current state of plant biotechnology, and to evaluate implications that may impact potential BW threats. The mechanism of xenogenic gene expression in plants is reviewed, along with examples of vaccines and antibodies which have been produced in plants. The economics and virtually unlimited quantities of material that can be produced, are the principle advantages of this eucaryotic expression system. The potential to use plants as a production system for biological toxins is a concern that warrants more detailed analysis.

Résumé

Dans ce rapport, nous avons voulu faire le point sur l'état actuel de nos connaissances en biotechnologie végétale et voir ce que cette discipline peut apporter pour contrer d'éventuels moyens de guerre biologiques. Nous examinons le mécanisme de l'expression de gènes hétérologues chez les végétaux ainsi que des exemples de vaccins et d'anticorps fabriqués par des plantes. Essentiellement, ce système d'expression eucaryote est intéressant pour ses avantages économiques et pour les quantités pratiquement illimitées de produit qu'il permet de fabriquer. Vu le potentiel que possèdent les plantes pour la fabrication de toxines biologiques, il est indiqué d'examiner cette question plus à fond.

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Executive summary

Plants are gaining recognition as an expression system for the production of a wide range of products. This report details the current advancements in the area of plant biotechnology, and their implications with regard to BW defence. Plant biotechnology has potential as a production system for vaccines and biologicals, as well as the large scale production of biological toxins for offensive use by rogue states.

Plant Genetic Engineering

Production of transgenic plants has progressed rapidly over the last decade. The procedure has become relatively simple and is capable of creating plants that produce an increasingly wide diversity of heterologous products at levels that can exceed 1% of the total plant protein. Several procedures available to transfer heterologous genes into a wide range of plant species including: *Agrobacterium tumefaciens* mediated transformation; direct uptake of naked DNA by plant protoplasts in the presence of poly-L-ornithine or polyethylene glycol (PEG); high velocity particle bombardment; and the use of infectious virus clones.

One of the best examples demonstrating the potential of plant genetic engineering is evident from one particular area, virus resistance strategies. The potential targets for disruption of the virus infection process include entry, disassembly, translation, replication, encapsidation, movement, and transmission.

Expression of Animal Proteins

Molecular farming is the production of recombinant proteins in plants. It has the potential to provide virtually unlimited quantities of recombinant antibodies for use as diagnostic and therapeutic tools in both health care and the life sciences. One application that has been explored is the use of transgenic plants to produce vaccines. Transgenic tobacco and potato plants were made by incorporation of genes encoding *Escherichia coli* heat-labile enterotoxin (LT-B). Mice immunized by gavage produced serum and gut mucosal anti-LT-B immunoglobulins that neutralized the enterotoxin in cell protection assays. Plants have also produced complex eucaryotic viral proteins including those from hepatitis B virus, human immunodeficiency virus type 1, foot and mouth disease virus, human cytomegalovirus and rabies virus.

An example of the plant's ability to correctly process molecules is the production of functional antibodies. Transgenic *Nicotiana tabacum* plants have been developed that express a murine monoclonal antibody kappa chain, a hybrid immunoglobulin A-G heavy chain, a murine joining chain and a rabbit secretory component. These chains were assembled into a functional, high molecular weight secretory immunoglobulin that recognized the native streptococcal antigen, a cell surface adhesion molecule. The ability to produce secretory monoclonal antibodies in plants offers the opportunity for the development of an inexpensive method of mucosal immunoprotection.

The production of recombinant proteins for therapeutic use in transgenic plants has become an alternative to the isolation of these molecules from natural or other recombinant sources. Plant bioreactors offer the possibility inexpensive, large scale production of recombinant proteins. Several laboratories are involved in the commercial production of the first recombinant products, such as antibodies or recombinant human hemoglobin from transgenic plants. Other proteins that have been successfully expressed include leech anticoagulant protein - hirudin, human serum albumin, interferon- β , erythropoietin, human growth hormone and human blood coagulation factors (including Factor VIII and Factor IX). This represents additional proof of the utility of plant expression since these factors are often quite large and require extensive post-translational modifications such as glycosylation, γ -carboxylation of specific glutamic acid residues, and β -hydroxylation of aspartic acid residues.

Conclusion

Plants represent a fascinating alternative eukaryotic expression system for the production of xenogenic products. They have enormous potential for the production of a wide variety of molecules, including vaccines and antibodies for diagnostics and therapeutics. Plant protein processing has not been an obstacle to producing complex heterologous proteins with specific activities equivalent to that obtained with other systems. Relative to the cost of conventional fermentation, plant genetic engineering potentially represents an inexpensive method of producing biologicals. On the downside, they represent a system for the potential development of products that are highly toxic to other systems (ie neurotoxins).

In summary, eucaryotic toxins (i.e., spider venoms, saxitoxin, etc.) that were not considered a potential BW threat due to limitations in the ability to produce the proteins, must be re-evaluated in light of the rapid advances in the field of plant biotechnology.

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Sommaire

Les végétaux sont de plus en plus reconnus comme systèmes d'expression pour la fabrication de toutes sortes de produits. Dans ce rapport, nous voyons en détail les derniers progrès de la biotechnologie végétale et leur retentissement sur nos moyens de défense contre les armes biologiques. Avec la biotechnologie végétale, les plantes peuvent être utilisées non seulement pour la fabrication de vaccins et de produits biologiques, mais aussi pour la production à grande échelle de toxines biologiques destinées à servir aux fins offensives des états hors-la-loi.

Génie génétique des végétaux

La production de plantes transgéniques a fait de grands progrès depuis une dizaine d'années. Les méthodes sont devenues relativement simples et permettent de créer des plantes capables fabriquer une gamme de plus en plus variée de produits hétérologues en des quantités dépassant parfois 1 % de la teneur totale de la plante en protéines. Plusieurs méthodes peuvent être utilisées pour introduire des gènes hétérologues chez des plantes d'espèces très variées; signalons entre autres la transformation par *Agrobacterium tumefaciens*; l'intégration d'ADN nu directement par des protoplastes végétaux en présence de poly-L-ornithine ou de polyéthylèneglycol (PEG), le bombardement de particules à grande vitesse et l'utilisation de clones de virus infectieux.

Les applications du génie génétique végétal aux mécanismes de résistance contre les virus illustrent avec le plus d'éloquence le potentiel de la génétique. Pour perturber le processus de l'infection virale, plusieurs éléments peuvent être ciblés : la pénétration, le désassemblage, la traduction, la réplication, l'encapsulation, le déplacement et la transmission.

Expression de protéines animales

L'agriculture moléculaire consiste à faire produire des protéines de recombinaison à des plantes. Grâce à cette technologie, on pourrait arriver à fabriquer des quantités pratiquement illimitées d'anticorps recombinants à des fins thérapeutiques et diagnostiques pour la santé et les sciences de la vie. L'utilisation de plantes transgéniques pour fabriquer des vaccins est l'une des applications qui ont été étudiées. On a mis au point des variétés transgéniques de tabac et de pomme de terre en y introduisant les gènes de l'entérotoxine thermolabile (LT-B) de *Escherichia coli*. Chez des souris immunisées par gavage, le sérum et les muqueuses intestinales renfermaient des immunoglobulines anti-LT-B qui ont neutralisé l'entérotoxine dans des épreuves de protection cellulaire. Des végétaux ont aussi été utilisés pour produire des protéines virales eucaryotes complexes, notamment du virus de l'hépatite B, de l'immunodéficience humaine de type 1, de la fièvre aphteuse et de la rage, ainsi que du cytomégalo virus humain.

La production d'anticorps fonctionnels montre que les plantes sont capables de traiter adéquatement les molécules. Ainsi, on a mis au point des sujets transgéniques de *Nicotiana tabacum* qui produisent une chaîne kappa d'anticorps monoclonal murin, une chaîne lourde d'immunoglobuline A-G hybride, une chaîne de raccordement murine et une composante

sécrétoire de lapin. Ces chaînes ont été assemblées en une immunoglobuline sécrétoire de poids moléculaire élevé qui est fonctionnelle et qui reconnaît l'antigène streptococcal natif, une molécule d'adhésion à la surface cellulaire. La fabrication d'anticorps monoclonaux sécrétoires chez les plantes ouvre la possibilité de la mise au point de moyens bon marché d'obtenir une immunoprotection par les muqueuses.

De nos jours, certaines protéines d'usage thérapeutique qu'on obtenait autrefois en les isolant de sources naturelles ou recombinantes peuvent être fabriquées par des plantes transgéniques. Les bioréacteurs végétaux offrent la possibilité de produire des protéines recombinantes à grande échelle et à peu de frais. Plusieurs laboratoires interviennent dans la production commerciale des premiers produits de recombinaison (anticorps et hémoglobine humaine) par des plantes transgéniques. Parmi les autres protéines qu'on a réussi à faire exprimer chez des plantes, signalons l'hirudine, l'anticoagulant de la sangsue, l'albumine sérique humaine, l'interféron- β , l'érythropoïétine, l'hormone de croissance humaine et les facteurs de coagulation sanguins humains (y compris les facteurs VIII et IX). La production de telles substances apporte une preuve de plus de l'utilité de l'expression de gènes chez les végétaux, car il s'agit dans bien des cas de composés d'assez grande taille qui nécessitent après la traduction d'importantes modifications comme la glycosylation, la carboxylation de certains résidus d'acide glutamique et l'hydroxylation de résidus d'acide aspartique.

Conclusion

Les plantes sont un système d'expression eucaryote à grand potentiel pour la production de toute une variété de molécules dont des vaccins et des anticorps d'usage diagnostique et thérapeutique. Le traitement des protéines d'origine végétale n'a pas freiné la production de protéines hétérologues complexes ayant des activités spécifiques équivalentes à celles des produits obtenus avec d'autres systèmes. Par comparaison au coût de la fermentation classique, le génie génétique des végétaux représente un moyen peu coûteux de fabriquer des produits biologiques. D'un autre côté, ce système pourrait aussi servir à faire des produits très toxiques (c.-à-d. des neurotoxines).

Bref, vu la rapidité des progrès de la biotechnologie végétale, il y a lieu de revoir la situation des toxines d'eucaryotes (c.-à-d. le venin d'araignée, la saxitoxine, etc.), qui jusqu'ici n'étaient pas considérées comme des armes biologiques en raison de difficultés de production.

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Introduction

As the mystique of genetically modified foods comes to the forefront of public interest, the extent of genetic engineering in plants has become apparent. Plants are gaining recognition as expression systems for the production of a wide range of proteins. This report details the current advancements in the area of plant biotechnology, and its implications in regards to BW defence in its potential as a production system for vaccines and biologicals. Due to the vast number of eucaryotic proteins that have been functionally expressed, examination of plant systems for the large scale production of biological toxins should also be undertaken.

Plant disease

Although it is outside the scope of this report, the potential biological attack on agricultural products (plants and animals) could be economically disastrous to a nation. There is considerable evidence that crop diseases can inflict devastation on a scale that rivals military invasion of a population (Rogers et al. 1999). There are many pathogens that cause considerable disease in the four major world crops, cereals, maize, rice and potatoes. For example, the late blight epidemic of potatoes in 1845 caused the Irish famine that killed or displaced several million people. A crop epidemic initiated by a natural pathogen may appear as a natural event and reduce the repercussion that an aggressor may experience. Psychologically an overt assault directed towards plants rather than people may be relatively acceptable.

Plant genetic engineering

A new class of potential biological weapons is emerging with the development of transgenic plants and the expression of heterologous proteins. Many plants produce natural products that are extremely toxic to humans and mammals, and it is this ability, combined with the seemingly unlimited diversity of heterologous products that can now be engineered into transgenic plants, that needs to be considered. For example, many plants produce numerous highly toxic glycoalkaloids and lectins (i.e., ricin) and it is this unique ability of eukaryotic processing, potentially making military significant levels of desired product, that may be exploited. The advantage of using plants to produce heterologous proteins is that they are usually unaffected by substances toxic to microbes, mammals and humans. Production of transgenic plants has progressed rapidly over the last decade. The procedure has become relatively simple and capable of creating plants that produce an increasingly wide diversity of heterologous products at levels that exceed 1% of the total plant protein.

Mechanism of gene transfer

There are several procedures available to transfer foreign DNA into plants. However, *Agrobacterium tumefaciens* mediated transformation is the most common and efficient procedure (Fraley et al. 1985). *Agrobacterium tumefaciens* is a soil phytopathogen that genetically transforms host cells, causing crown gall tumors, an agronomically important disease that affects most dicotyledonous plants. The *Agrobacterium*-plant cell interaction is the only known natural example of DNA transport between kingdoms. Inside this process, DNA is transported from wild-type *Agrobacterium* into the plant cell nucleus. Expression of this transferred DNA (T-DNA) results in neoplastic growths (tumors) on the host plant. The wild-type T-DNA carries genes involved in the synthesis of plant growth hormones and the production of opines, tumor-specific compounds formed by the condensation of an amino acid with a keto acid or a sugar. It is the production of growth hormones in the transformed host cells that induces the formation of tumors. These tumors then synthesize opines, a major carbon and nitrogen source for *Agrobacterium*. *Agrobacteria* are usually classified based on the type of opines specified by the bacterial T-DNA, the most common strains being octopine or nopaline specific. Opine import into, and the subsequent catabolism within, the bacterial cell requires specialized enzymes. Since these enzymes are encoded by the *Agrobacterium* tumor-inducing (Ti) plasmid, practically no other soil microorganisms can metabolize opines, creating a favorable biological niche for *Agrobacterium*.

Three genetic components of *Agrobacterium* are required for plant cell transformation. The first component is the T-DNA, which is actually transported from the bacterium to the plant cell. The T-DNA is a discrete segment of DNA located on the 200 kb Ti plasmid of *Agrobacterium*; it is delineated by two 25 base pair (bp) imperfect direct repeats known as the T-DNA borders. The second component is the 35 kb virulence (vir) region, also located on the Ti plasmid, which is composed of seven major loci (virA, virB, virC, virD, virE, virG, and virH). The protein products of these genes, termed virulence (Vir) proteins, respond to the specific compounds secreted by the wounded plant to generate a copy of the T-DNA and mediate its transfer into the host cell. The third component is the suite of chromosomal virulence (chv) genes, located on the *Agrobacterium* chromosome. Chv genes are involved in bacterial chemotaxis toward and attachment to the wounded plant cell. Because the T-DNA element is defined by its borders, the coding region of the wild-type T-DNA can be replaced by any DNA sequence without any effect on its transfer from *Agrobacterium* to the plant. Thus, *Agrobacterium* is often used to produce transgenic plants expressing genes of interest.

For many years, direct uptake of naked DNA by plant protoplasts has been the sole alternative to *Agrobacterium tumefaciens*-mediated gene transfer. The first experiments demonstrating direct gene transfer included the delivery of isolated plasmid DNA to protoplasts of petunia and tobacco in the presence of poly-L-ornithine or polyethylene glycol (PEG). During the following years, protoplast transformation mediated by PEG or electroporation was substantially simplified, and their efficiency in model systems was increased by several orders of magnitude. The production of transgenic plants via direct gene transfer to protoplasts depends on protoplast-to-plant regeneration and on efficient selection systems for transgenic clones. Early gene transfer experiments focused on protoplasts of Solanaceae species that are easily regenerated, and on the use of the bacterial gene for neomycin phosphotransferase (npt II), conferring antibiotic resistance to transformed clones. During the past few years, protoplast-to-plant regeneration was achieved for many other plant species.

High velocity microprojectiles may be used to deliver nucleic acids into living cells. The technique of particle bombardment (otherwise known as biolistics, microprojectile bombardment, particle acceleration, etc.) has been shown to be the most versatile and effective way for the creation of many transgenic organisms, including microorganisms, mammalian cells, and a large number of plants species. An estimated two hundred papers have been published on various aspects of the technique. Several advantages make microprojectile bombardment the method of choice for engineering crop species:

- a) Transformation of organized tissue: The ability to engineer organized and potentially regenerable tissue permits introduction of foreign genes into elite germplasm.
- b) Universal delivery system: Transient gene expression has been demonstrated in numerous tissues representing many different species. In particular cases in which recovery of transgenic plants has not been reported, this deficit is more due to the lack of a favorable tissue culture response than the DNA delivery method.
- c) Transformation of recalcitrant species: Engineering of important agronomic crops such as soybean, cotton, maize, rice, etc. has been restricted to a few non-commercial varieties when conventional methods are used. Particle bombardment technology allowed recovery of transgenic plants from many commercial cultivars.
- d) Study of basic plant developmental processes: By utilizing chromogenic markers, it is possible to study developmental processes and also clarify the origin of germline in regenerated plants.

Infectious virus clones represent an emerging expression strategy that may produce relatively high amounts of heterologous proteins, possibly up to 10 to 30% of total plant protein. A full-length cDNA of potato leafroll virus (PLRV) was constructed and examined *in vivo* for its biological activities by transient expression experiments with plasmid DNA or *in vitro* transcribed RNA (Prüfer et al. 1997, Kawchuk unpublished). In addition, PLRVfl cDNA was stably introduced into the genome of potato plants by *Agrobacterium*-mediated leaf disc transformation. Both transient and stable expression of PLRVfl resulted in the synthesis of genomic and subgenomic PLRV RNAs. Transgenic plants accumulated virus and displayed the typical symptoms of PLRV infection.

Genetic alterations of plants

There is a great diversity of products and modifications that can be achieved through plant transformation. Genetic engineering of new storage oils and fats has produced oil crop plants with fatty acid compositions unattainable by plant breeding alone. The combination of classical breeding methods with molecular techniques provides new ways for designing oils for food and nonfood uses (Topfer et al. 1995). Alterations in the position and number of double bonds, variation in fatty acid chain length, and the introduction of desired functional groups have already been achieved in model systems. Short-term prospects include crops such as rapeseed or soybean engineered to have greater than 70 to 80 percent medium-chain fatty acids by content, greater than 90 percent oleic acid, high erucic acid content, and engineered to form ricinoleic acid in seed storage tissues.

Plants are also capable of producing heterologous products that are quite unrelated to any endogenous molecules. Polyhydroxybutyrate (PHB), a high molecular weight polyester, is accumulated as a storage carbon in many species of bacteria and is a biodegradable thermoplastic. To produce PHB by genetic engineering in plants, genes from the bacterium *Alcaligenes eutrophus* that encoded the two enzymes required to convert acetoacetyl-coenzyme A to PHB were placed under transcriptional control of the cauliflower mosaic virus 35S promoter and introduced into *Arabidopsis thaliana* (Poirer et al. 1992). Transgenic plant lines that contained both genes accumulated PHB as electron-lucent granules in the cytoplasm, nucleus, and vacuole; the size and appearance of these granules were similar to the PHB granules that accumulate in bacteria.

Through gene silencing, companies can now ensure that genetically modified plants produce sterile seeds - a feat that will keep farmers coming back for fresh seed each year (Service 1999). Companies say the innovation will prevent the transfer of traits such as herbicide resistance to weedy species and is needed to safeguard their investments in improved plant varieties. Pollen dispersal can be an effective means of distributing a gene that could impact health and environment.

Plants have been engineered to produce many pesticides such as the *Bacillus thuringiensis* (Bt) endotoxin (Huang et al. 1999). Although plants transformed with genetic material from the bacterium are generally thought to have negligible impact on non-target organisms, Bt corn plants might represent a risk because most hybrids express the Bt toxin in pollen, and corn pollen is dispersed over a distance of at least 60 metres by wind. Corn pollen is deposited on other plants near corn fields and can be ingested by the non-target organisms that consume these plants. In a laboratory assay, larvae of the monarch butterfly, *Danaus plexippus*, reared on milkweed leaves dusted with pollen from Bt corn, ate less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with untransformed corn pollen or on leaves without pollen.

Plants may serve as an alternative system for the production of highly toxic substances (Rao et al 1998). Recent work by Dr. Pusztai at the Rowett Research Institute concentrated on tubers from genetically modified (GM)-potato lines expressing the gene of snowdrop (*Galanthus nivalis*) bulb lectin (GNA). Many lectins (i.e., ricin) have extremely high levels of toxicity. The lectin-expressing GM plants were evaluated to determine the analytical, nutritional and immunological impact of GNA-GM-potatoes fed to rats. Data suggests that the consumption by rats of transgenic potatoes expressing GNA had significant detrimental effects on organ development, body metabolism and immune function. Parallels can be drawn between the current production of biological toxins to control insects in plants, to the potential production of toxins that could be used in biological warfare (Ewen and Pusztai 1999).

Engineering resistance to disease

One of the best examples for demonstrating the capabilities of plant genetic engineering is evident from one particular area, virus resistance strategies. Potato (*Solanum tuberosum* ssp. *tuberosum* L.), one of the most important food crops in the world, is especially important in this regard since it is particularly subject to virus diseases because vegetative propagation allows pathogens several years to accumulate over successive generations. It is, therefore critical, that the introduction, accumulation and transmission of viruses in vegetatively propagated crops be prevented or attenuated. Control of viruses in potato has relied on management practices, greatly assisted by sensitive immunological and nucleic acid detection methods. Tissue culture involving the isolation of virus-free axillary meristems is used, sometimes in combination with thermotherapy or chemotherapy, to eradicate viruses and produce the earliest generation of seed potatoes (Mellor and Stace-Smith 1977). Stringent regulations and certification ensures that virus levels do not exceed prescribed levels in each subsequent generations. These measures have kept the incidence of potato viruses relatively low in early generation seed potatoes but there continues to be a need to identify improved cost-effective and environmentally acceptable virus control, especially for the production of later potato generations.

Resistance, the ability to prevent virus infection or reduce virus titres, is the usual plant response to most viruses. Interestingly, a virus can also induce resistance in a plant against symptoms produced by related viruses, a phenomenon referred to as cross-protection (McKinney 1929). Hamilton (1980) suggested that similar virus-induced resistance could be obtained by transferring a portion of a viral genome into a plant. Alternatively, heterologous genes from sources other than a virus could provide engineered resistance to plant viruses. Fraley et al. (1983) constructed chimeric genes that functioned as dominant selectable markers in plant cells. Analysis of progeny from transformed tobacco plants indicated that the chimeric kanamycin resistance gene was inherited and was expressed as a dominant Mendelian trait (Horsch et al. 1985). The solanaceous species are the host plants most frequently used to engineer resistance, being relatively simple to transform.

Strategies for genetic engineering of resistance to plant viruses increase as our understanding of virus infection and host-pathogen interactions improves. The potential targets for disruption of the virus infection process include entry, disassembly, translation, replication, encapsidation, movement, and transmission. Since 1986, there have been more than 100 reports of plants with genetically engineered virus resistance. Several mechanisms that are not necessarily mutually exclusive have been proposed to explain the resistance observed with the various strategies. Reviews related to engineering resistance to plant viruses include Beachy et al. (1990), Hull and Davies (1992), Fitch and Beachy (1993), Scholtof et al. (1993), Wilson (1993), Hackland et al. (1994), Grunet (1995), Pappu et al. (1995), Baulcombe (1996) and Palukaitis and Zaitlin (1997).

Genetically engineered plant virus resistance was first reported by Powell et al. (1986). A cDNA clone of the tobacco mosaic tobamovirus (TMV) coat protein gene was constitutively expressed in *Nicotiana tabacum* and plants that accumulated coat protein developed no disease symptoms, or showed delayed symptom development when inoculated with TMV. The first report of genetically engineered plant virus resistance in potato described the use of the coat protein gene of potato potexvirus X (PVX) to delay symptom development and

reduce virus accumulation (Hoekema et al. 1989). Genetic engineering of plant virus resistance using the coat protein gene is the most widely examined strategy and has been reported for plant viruses with single-stranded positive- and negative-sense RNA, double-stranded RNA, and DNA genomes (Table 1). Protection has been observed for plant viruses with differing particle morphology, genome organization, host range and vector specificity. In general, expression of a virus coat protein gene reduces symptom expression, slows virus movement, and reduces titres of closely related viruses. Resistance often depends on the level of coat protein expression, as observed with alfalfa mosaic alfamovirus (AIMV) (Loesch-Fries et al. 1987), plum pox potyvirus (PPV) (Ravelonandro et al. 1993), PVX (Hemenway et al. 1988, Hoekema et al. 1989), rice stripe tenuvirus (RSV) (Hayakawa et al. 1992), TMV (Powell et al. 1990), and tomato yellow leaf curl geminivirus (TYLCV) (Kumk et al. 1994). Variability of resistance has also been observed within individual transformed lines, since the random insertion of the coat protein gene into the genome can influence expression levels. Plants expressing higher levels of coat protein exhibited a greater level of protection.

Table 1. Genetically engineered virus resistance using viral coat protein genes.

Virus group	Virus	Abbreviation	Plant host	Reference
Alfavirus	Alfalfa mosaic virus	AIMV	Tobacco	Loesch-Fries et al. 1987
			Tobacco	van Dun et al. 1987
			Tobacco, tomato	Turner et al. 1987
			Alfalfa	Hill et al. 1991
Carlavirus	Potato virus S	PVS	Tobacco	Taschner et al. 1994
Cucumovirus	Cucumber mosaic virus	CMV	<i>N. debneyi</i> *	MacKenzie and Tremaine 1990
			Potato	MacKenzie et al. 1991
			Tobacco	Cuzzo et al. 1988
			Tobacco	Quemada et al. 1991
			Tobacco	Namba et al. 1991
			Tobacco	Nakajima et al. 1993
			Cucumber	Gonsalves et al. 1992
			Tobacco	Yin et al. 1992
Geminivirus	Tomato yellow leaf curl virus	TYLCV	Squash	Tricoli et al. 1995
			Tomato	Kunk et al. 1994
			<i>N. benthamiana</i> *	Nons et al. 1996
Ilavrus	Tobacco streak virus	TSV	Tobacco	van Dun et al. 1988
Luteovirus	Potato leafroll virus	PLRV	Potato	Kawchuk et al. 1990
			Potato	Kawchuk et al. 1991
			Potato	van der Wilk et al. 1991
			Potato	Barker et al. 1992
			Tobacco	Barker et al. 1993
			Potato	Barker et al. 1994
			Potato	Prenting et al. 1995
			Potato	Derrick and Barker 1997
			Potato	Graham et al. 1997
			Potato	Kawchuk et al. 1997
Nepovirus	Arabis mosaic virus	ArMV	Tobacco	Bartoli et al. 1992
	Grapevine chrome mosaic virus	GCMV	Tobacco	Brault et al. 1993
	Grapevine fanleaf virus	GFLV	Tobacco	Bardonnnet et al. 1994
	Tomato ringspot virus	TomRSV	Tobacco, <i>N. benthamiana</i> *	Yepes et al. 1996
Pomovirus**	Potato mop top virus	PMTV	<i>N. benthamiana</i> *	Raavy et al. 1995
Potyvirus	Cymbidium mosaic virus	CymV	<i>N. benthamiana</i> *	Chia et al. 1992
	Potato aucuba mosaic virus	PAMV	Tobacco	Lederc and Abou-Haidar 1995
	Potato virus X	PVX	Tobacco	Hemenway et al. 1983
			Potato	Hoekema et al. 1989
			Potato	Kaniewski et al. 1990
			Potato	Lawson et al. 1990
			Potato	Jongardijk et al. 1992
			Tobacco	Spillens et al. 1997

* *Nicotiana benthamiana* and *Nicotiana debneyi*.

** Previously classified as a Furovirus.

Table 1. Genetically engineered virus resistance using viral coat protein genes (continued).

Virus group	Virus	Abbreviation	Plant host	Reference
Potyvirus	Bean yellow mosaic virus	BYMV	<i>N. benthamiana</i> *	Hammond and Kamo 1993
	Lettuce mosaic virus	LMV	<i>N. benthamiana</i> *	Hammond and Drenth 1997
	Maze dwarf mosaic virus	MDMV	Tobacco	Dinant et al. 1993
	Papaya ringpot virus	PRSV	Corn	Murray et al. 1993
			Tobacco	Ling et al. 1991
			Papaya	Fitch et al. 1992
	Plum pox virus	PPV	<i>N. benthamiana</i> *, <i>N. clevelandi</i>	Regner et al. 1992
			<i>N. benthamiana</i> *	Ravelonandro et al. 1993
	Potato virus Y	PVY	Potato	Lawson et al. 1990
			Potato	Kaniewski et al. 1990
			Tobacco	Smith et al. 1994
			Potato	Smith et al. 1995
			Tobacco	Marcos and Beachy 1997
	Peanut stripe virus	PsTV	<i>N. benthamiana</i> *	Cassidy and Nelson 1995
	Soybean mosaic virus	SMV	Tobacco	Stark and Beachy 1989
Tenuvirus**	Tobacco etch virus	TEV	Tobacco	Lindbo et al. 1993
	Watermelon mosaic virus	WMV	<i>N. benthamiana</i> *	Namba et al. 1992
		WMV2	Squash	Fuchs and Bonasves 1995
			Squash	Tricoli et al. 1995
	Zucchini yellow mosaic virus	ZYMV	<i>N. benthamiana</i> *	Namba et al. 1992
			Melon, tobacco	Pang and Grunet 1993
			Squash	Fuchs and Bonasves 1995
			Squash	Tricoli et al. 1995
	Rice stripe virus	RSV	Rice	Hayakawa et al. 1992
	Pepper mild mottle virus	PMMV	Pepper	Lim et al. 1997
	Tobacco mosaic virus	TMV	Tobacco	Powell et al. 1988
			Tomato	Nelson et al. 1988
			Tobacco	Neydat and Beachy 1989
			Tobacco	Powell et al. 1990
			Tobacco	Waniewski et al. 1990
Tobamovirus			Tobacco	Reimann-Philipp and Beachy 1993
			Tobacco	Clark et al. 1995a
			Tobacco	Clark et al. 1995b
			Tobacco	Bendahmane et al. 1997
			Tobacco	Marcos and Beachy 1997
	Tomato mosaic virus	ToMV	Tomato	Sanders et al. 1992
	Tobacco rattle virus	TRV	<i>N. benthamiana</i> *	van Dun et al. 1987
			Tobacco	van Dun and Bol 1988
			Tobacco	Angenent et al. 1990
			Tobacco	Glenn et al. 1991
			Tobacco	de Haan et al. 1992
			Tobacco	MacKenzie and Ellis 1992
			Tobacco	Pang et al. 1992
			Lettuce	Pang et al. 1995
			Tobacco	Prins et al. 1995
Tospovirus	Tomato spotted wilt virus	TSWV	<i>N. benthamiana</i> *	Vera et al. 1995
			Tobacco	Prins et al. 1995
	Tomato chlorotic spot virus	TCSV	Tobacco	Prins et al. 1995
	Groundnut ringpot virus	GRSV	Tobacco	Prins et al. 1995

* *Nicotiana benthamiana* and *Nicotiana clevelandi*

** No member of this group has been reported to infect potato.

Several other virus genes have been used to engineer resistance including the replicase, movement protein, and protease genes (Table 2). Unlike the expression of coat protein, the development of resistance with nonstructural genes usually requires the expression of an incompatible or defective protein that interferes with virus replication, movement, or maturation of virus proteins. Resistance levels varied between transformed lines but were frequently higher with the replicase gene than that observed using coat protein genes, but were extremely specific for the virus or isolate from which the replicase was derived.

Cell-to-cell movement of viruses can involve a virus encoded movement protein that interacts with the plasmodesmata to increase the size exclusion limit (Deom et al. 1992, Wolf et al. 1989). Tobacco plants transformed with a defective TMV movement protein gene or with the movement protein gene from BMV, a virus that cannot move within tobacco, are resistant to TMV (Lapidot et al. 1993, Malysenko et al. 1993). Similarly, Tacke et al. (1996) expressed modified movement proteins of PLRV in potato and observed broad-spectrum resistance to PLRV, as well as to unrelated PVX, and PVY. Resistance to PVX also was observed in potato plants expressing modified movement proteins of PVX (Seppänen et al. 1997). The broad-spectrum resistance induced by movement protein to viruses from five groups may be a consequence of defective or heterologous movement proteins competing with functional movement proteins for a limited number of target sites within the plasmodesmata.

Several plant viruses encode polyproteins that are processed by virus encoded proteases. For example, the potyviruses produce a single polyprotein that is cleaved into mature virus proteins by three virus encoded proteases (Dougherty and Carrington 1988). When Vardi et al. (1993) transformed tobacco with the PVY N1a protease, they observed that 2 of 50 plants were resistant to PVY. The authors suggested that changes during cloning may have produced dysfunctional protein. Similarly, resistance to TMV in tobacco was conferred by genetically modified proteases (Maiti et al. 1993).

Virus untranslatable nucleic acids have been exploited in engineering resistance to viruses from nine groups (Table 3). Antisense RNA and untranslatable sense RNA sequences derived from different regions of the viral genome have been used, including coat protein (Cuozzo et al. 1988, Hemenway et al. 1988, Rezaian et al. 1988, Kawchuk et al. 1991, van der Wilk et al. 1991, Smith et al. 1994), movement protein (Tacke et al. 1996, Prins et al. 1997), or replicase genes (Day et al. 1991, Mueller et al. 1995, Swaney et al. 1995). Potato transformed with antisense RNA of the PLRV coat protein gene (Kawchuk et al. 1991, van der Wilk et al. 1991), a mutated sense RNA of the PLRV movement protein gene unable to produce detectable amounts of protein (Tacke et al. 1996), or untranslatable sense and antisense constructs of the PVY coat protein gene (Smith et al. 1995), were resistant to the viruses from which the sequences were derived. The resistance is believed to involve an interaction of complementary virus and transgene sequences that disrupt translation, replication, stability, or access of host molecules. Sequences complementary to virus RNA have been developed to target sequence-specific ribozymes that catalyze biochemical restriction of single-stranded RNA at a specific sequence. Transgenic plants expressing a ribozyme specific for TMV or CMV RNA reduced virus titres (de Feyter et al. 1996, Kwon et al. 1997). However, antisense RNA was reported to be as effective as the ribozyme (de Feyter et al. 1996).

Table 2. Genetically engineered resistance using non-structural virus genes.

Virus group	Virus	Abbreviation	Plant host	Reference
Replisase				
Alfavirus Cucumovirus	Alfalfa mosaic virus	AIMV	Tobacco	Braderode et al. 1995
	Cucumber mosaic virus	CMV	Tobacco	Cuozzo et al. 1988
			Tobacco	Anderson et al. 1992
			Tobacco	Carr et al. 1994
			Tobacco	Zaitlin et al. 1994
			Tobacco	Hellwald and Pakulatis 1995
			Tobacco	Nguyen et al. 1996
Geminivirus	African cassava mosaic virus	ACMV	<i>N. benthamiana</i> *	Wintermantel et al. 1997
	Tomato yellow leaf curl virus	TYLCV	<i>N. benthamiana</i> *	Hong and Stanley 1996
Potyvirus	Potato virus X	PVX	Tobacco	Nons et al. 1996
			Tobacco, <i>N. clevelandi</i> *	Braun and Hemenway 1992
Potyvirus	Potato virus Y	PVY	Tobacco	Longstaff et al. 1993
	Plum pox virus	PPV	<i>N. benthamiana</i> *	Audy et al. 1994
			<i>N. benthamiana</i> *	Guo et al. 1996
Tobamovirus	Tobacco etch virus	TEV	<i>N. benthamiana</i> *	Wittner et al. 1996
	Tobacco mosaic virus	TMV	Tobacco	Swaney et al. 1995
			Tobacco	Golembocki et al. 1990
			Tobacco	Carr and Zaitlin 1991
			Tobacco	Donson et al. 1993
		Pepper mild mottle virus	PMMV	<i>N. benthamiana</i> *
			<i>N. benthamiana</i> *	Tenllado et al. 1996
Tobravirus	Pee early browning virus	PEBV	<i>N. benthamiana</i> *	MacFarlane and Davies 1992
Tombusvirus	Cymbidium ringspot virus	CyRSV	<i>N. benthamiana</i> *	Rubino et al. 1993
			<i>N. benthamiana</i> *	Rubino and Rubino 1995
Tospovirus	Tomato spotted wilt virus	TSWV	Tobacco	Prins et al. 1996
Movement				
Comovirus	Cowpea mosaic virus	CPMV	<i>N. benthamiana</i> *	Sijen et al. 1995
Luteovirus	Potato leafroll virus	PLRV	Potato	Tacke et al. 1996
Potyvirus	Potato virus X	PVX	Potato	Seppänen et al. 1997
			Tobacco	Areas et al. 1996
Tobamovirus	White clover mosaic virus	WCIMV	<i>N. benthamiana</i> *	Beck et al. 1994
	Tobacco mosaic virus	TMV	Tobacco	Malyshenko et al. 1993
			Tobacco	Lapidot et al. 1993
			Tobacco	Cooper et al. 1995
Tospovirus	Tomato spotted wilt virus	YSWV	Tobacco	Areas et al. 1993
			Tobacco	Prins et al. 1997
Protease				
Potyvirus	Potato virus Y	PVY	Tobacco	Vardi et al. 1993
	Tobacco vein mottling virus	TMV	Tobacco	Malt et al. 1993

* *Nicotiana benthamiana* and *Nicotiana clevelandi*.

Table 3. Genetically engineered virus resistance using virus untranslatable nucleic acid sequences.

Virus group	Virus	Abbreviation	Plant host	Reference
Antisense RNA				
Cucumovirus	Cucumber mosaic virus	CMV	Tobacco	Cuozzo et al. 1988
Geminivirus	Tomato golden mosaic virus	TGMV	Tobacco	Rezaei et al. 1988
	Tomato yellow leaf curl virus	TYLCV	<i>N. benthamiana</i> *	Day et al. 1991
Luteovirus	Potato leafroll virus	PLRV	Potato	Bendahmane and Gronenborn 1997
			Potato	Kawchuk et al. 1991
Nepovirus	Tomato ringspot virus	TomRSV	Potato	van der Wilk et al. 1991
Potexvirus	Potato virus X	PVX	Tobacco, <i>N. benthamiana</i> *	Palucha et al. 1997
Polyvirus	Bean yellow mosaic virus	BYMV	Tobacco	Yepes et al. 1998
	Potato virus Y	PVY	Tobacco	Hemerway et al. 1988
Tobamovirus	Tobacco etch virus	TEV	<i>N. benthamiana</i> *	Mueller et al. 1995
	Zucchini yellow mosaic virus	ZYMV	Potato	Hammond and Karno 1993
	Tobacco mosaic virus	TMV	Tobacco	Smith et al. 1994
			Tobacco	Smith et al. 1995
Tospovirus	Tomato spotted wilt virus	TSWV	Melon, tobacco	Lindbo and Dougherty 1992
			Tobacco	Fang and Grunet 1993
			Tobacco	Powell et al. 1989
			Tobacco	de Feyter et al. 1998
			Tobacco	Pang et al. 1993
			Tobacco	Prins et al. 1997
Sense RNA				
Luteovirus	Potato leafroll virus	PLRV	Potato	Tacke et al. 1998
Potexvirus	Potato virus X	PVX	Tobacco	Mueller et al. 1995
Polyvirus	Potato virus Y	PVY	Tobacco	van der Vlugt et al. 1992
			Tobacco	Smith et al. 1994
			Potato	Smith et al. 1995
	Tobacco etch virus	TEV	Tobacco	Lindbo and Dougherty 1992
			Tobacco	Swaney et al. 1995
			Tobacco	Goodwin et al. 1998
Tobamovirus	Tobacco mosaic virus	TMV	Tobacco	Nelson et al. 1993
Tospovirus	Tomato spotted wilt virus	TSWV	Tobacco	de Haan et al. 1992
			Tobacco	Pang et al. 1992
			Tobacco	Pang et al. 1993
			<i>N. benthamiana</i> *	Pang et al. 1994
			<i>N. benthamiana</i> *	Pang et al. 1997
			Tobacco	Prins et al. 1997
			<i>N. benthamiana</i> *	Guo et al. 1998
Tymovirus	Turnip yellow mosaic virus	TYMV	Rapeseed	Zaccaroni et al. 1993
Ribozymes				
Cucumovirus	Cucumber mosaic virus	CMV	Tobacco	Kwon et al. 1997
Tobamovirus	Tobacco mosaic virus	TMV	Tobacco	de Feyter et al. 1998

* *Nicotiana benthamiana*

Virus satellites and defective interfering molecules are parasites that rely on the associated helper virus for replication and have been reported to attenuate virus symptoms and multiplication (Hillman et al. 1987, Collmer and Howell 1992, Roossinck et al. 1992). Transgenic plants expressing satellites or defective interfering sequences of viruses from five different groups have been developed (Table 4). Inoculation with the helper virus multiplies the transgene sequence, leading to a level of protection that is independent of inoculum levels and the expression level of the transgene sequence (Harrison et al. 1987, Jacquemond et al. 1988).

Full-length infectious virus clones of CMV or PVX, expressed by transgenic plants, conferred resistance to related viruses (Suzuki et al. 1996, Angell and Baulcombe 1997). Resistance may occur at several points during virus multiplication, analogous to the original cross-protection phenomenon reported by McKinney (1929). However, expression of a PLRV full-length clone was lethal in potato (Prüfer et al. 1997) and, as with satellites and defective interfering RNA, the potential application of replicating and complex constructs for developing resistance may be limited because of ecological concerns related to recombination and alteration of wild viruses.

Recently, heterologous proteins were identified that confer plant virus resistance (Lodge et al. 1993, Tavladoraki et al. 1993, Truve et al. 1993) (Table 5). Resistance to PLRV, PVX, and PVY was reported in potato and tobacco expressing a pokeweed ribosome-inhibiting antiviral protein (Lodge et al. 1993), and potato plants expressing a 2'-5' oligoadenylate synthetase, a component of the mammalian interferon-induced antiviral response, were resistant to PVX (Truve et al. 1993). Using the ACMV transactivated promoter confined expression of the ribosome-inhibiting protein and the virus to cells involved in the primary infection (Hong et al. 1996). Expression of a double-stranded RNA yeast ribonuclease in plants provided resistance to ToMV, CMV, and PVY. The ribonuclease is thought to attack the double-stranded RNA that occurs during replication of many plant viruses (Watanabe et al. 1995). *Nicotiana benthamiana* expressing single chain plantibodies (antibodies expressed in plants) specific for the coat proteins of artichoke mottled crinkle tombusvirus (AMCV) or TMV, exhibited virus specific resistance (Tavladoraki et al. 1993, Voss et al. 1995). Heterologous proteins represent a diverse strategy with enormous potential for virus control.

Strategies for engineering virus resistance have increased rapidly since the initial report of transformation with the TMV coat protein. Gene expression can be localized in specific tissues (Reimann-Philipp and Beachy 1993, Graham et al. 1997), optimized to coincide with the virus infection (Hong et al. 1996), and adjusted to achieve the highest possible levels of resistance. Furthermore, several strategies may be combined to increase the level and number of viruses to which the host is resistant. Yie et al. (1992) were able to increase resistance to CMV by expressing satellite RNA and the coat protein gene in tobacco. Barker et al. (1994) transformed PLRV resistant germplasm with the PLRV coat protein gene and reported the resistances were additive.

Table 4. Genetically engineered virus resistance using virus-replicated sequences.

Virus group	Virus	Abbreviation	Plant host	Reference
Satellites				
Cucumovirus	Cucumber mosaic virus	CMV	Tobacco	Harrison et al. 1987
			Tobacco	Jacquemond et al. 1988
			Tobacco	Yie et al. 1992
			Tomato	Saito et al. 1992
Nepovirus	Tobacco ringspot virus	TobRV	Tobacco	Gerlach et al. 1987
Umbravirus	Groundnut rosette virus	GRV	<i>N. benthamiana</i> *	Tatlianaky et al. 1998
Defective interfering sequences				
Geminivirus	African cassava mosaic virus	ACMV	<i>N. benthamiana</i> *	Stanley et al. 1990
	Beet curly top virus	BCTV	<i>N. benthamiana</i> *	Friedmann and Stanley 1994
			<i>N. benthamiana</i> *	Stenger 1994
Tombusvirus	Cymbidium ringspot virus	CyRSV	<i>N. benthamiana</i> *	Koller et al. 1993
Replicative virus clones				
Cucumovirus	Cucumber mosaic virus	CMV	Tobacco	Suzuki et al. 1996
Potexvirus	Potato virus X	PVX	Tobacco	Angell and Baucombe 1997

* *Nicotiana benthamiana*.

Table 3. Genetically engineered virus resistance using heterologous genes.

Virus group	Virus	Abbreviation	Plant host	Reference
Antiviral				
Geminivirus	African cassava mosaic virus	ACMV	<i>N. benthamiana</i> *	Hong et al. 1996
Luteovirus	Potato leafroll virus	PLRV	Potato, tobacco	Lodge et al. 1993
Potexvirus	Potato virus X	PVX	Potato	Moon et al. 1997
			Potato, tobacco	Lodge et al. 1993
			Potato	Truve et al. 1993
			Potato	Moon et al. 1997
			Tobacco	Turner et al. 1997
Potyvirus	Potato virus Y	PVY	Potato, tobacco	Lodge et al. 1993
			Potato	Moon et al. 1997
			Tobacco	Takahashi et al. 1997
Tobamovirus	Tobacco mosaic virus	TMV	Tobacco	
Plantibody				
Furovirus	Beet necrotic yellow vein virus	BNYVV	<i>N. benthamiana</i> *	Fecker et al. 1997
Tombusvirus	Artichoke mottled crinkle virus	AMCV	<i>N. benthamiana</i> *	Tavliodoraki et al. 1993
	Tobacco mosaic virus	TMV	Tobacco	Voes et al. 1995
Resistance gene				
Tobamovirus	Tobacco mosaic virus	TMV	Tobacco	Whitham et al. 1994
			Tobacco	Dinesh-Kumar et al. 1995
			Tobacco	Whitham et al. 1996
Ribonuclease				
Tobamovirus	Tomato mosaic virus	ToMV	Tobacco	Watanabe et al. 1995
Cucumovirus	Cucumber mosaic virus	CMV	Tobacco	Watanabe et al. 1995
Potyvirus	Potato virus Y	PVY	Tobacco	Watanabe et al. 1995

* *Nicotiana benthamiana*.

As outlined, the area of engineered virus resistance facilitates the incorporation and functional expression of genes from other organisms, such as antibodies and interferon effectors from mammals. In addition to potentially improving yield and quality, engineered virus resistance may reduce transmission by vectors (Table 6) and thereby reduce the use of pesticides. Regulatory approval has been granted by the US Department of Agriculture for the commercial release of papaya resistant to papaya ringspot potyvirus (PRSV) (Fitch et al. 1992) and squash resistant to CMV, WMV2 and ZYMV (Tricoli et al. 1995).

Table 6. Influence of genetic engineering on vector transmission.

Virus group	Virus	Vector	Plant host	Reference
Cucumovirus	Cucumber mosaic virus	Aphid	Tobacco	Jacquemond et al. 1988
			Tobacco	Quemada et al. 1991
			Cucumber	Gonsalves et al. 1992
			Tobacco	Zaitin et al. 1994
Gemmovirus	Tomato yellow leaf curl virus	Whitefly	Tomato	Kunik et al. 1994
Luteovirus	Potato leafroll virus	Aphid	Potato	Kawchuk et al. 1990
			Potato	Kawchuk et al. 1991
			Potato	van der Wilk et al. 1991
			Potato	Barker et al. 1992
			Potato	Presling et al. 1995
			Potato	Demck and Barker 1997
			Potato	Graham et al. 1997
			Potato	Kawchuk et al. 1997
			Potato, tobacco	Lodge et al. 1993
Pomovirus**	Potato mop top virus	Fungus	<i>N. benthamiana</i> *	Reevy et al. 1995
Potavirus	Potato virus X	Aphid	Potato	Lawson et al. 1990
Potyvirus	Potato virus Y	Aphid	Potato	Lawson et al. 1990
	Tobacco etch virus	Aphid	Tobacco	Lindbo and Dougherty 1992
			Potato, tobacco	Lodge et al. 1993
Tenuivirus***	Rice stripe virus	Planthopper	Rice	Hayakawa et al. 1992
Tospovirus	Tomato spotted wilt virus	Thrips	Tobacco	de Haan et al. 1992

* *Nicotiana benthamiana*.

** Previously classified as a Furovirus.

*** No member of this virus group has been reported to infect potato.

Expression of animal proteins

Molecular farming is the production of recombinant proteins in plants. It is intended to harness the power of agriculture to cultivate and harvest transgenic plants producing recombinant therapeutics, at low cost. Molecular farming has the potential to provide virtually unlimited quantities of recombinant antibodies for use as diagnostic and therapeutic tools in both health care and the life sciences. Importantly, recombinant antibody expression can be used to modify the inherent properties of plants, for example by using expressed anti-pathogen antibodies to increase disease resistance. Plant transformation is technically straightforward in model plant species and some cereals, and the functional expression of recombinant proteins can be rapidly analyzed using transient expression systems in intact or virally infected plants. Protein production can then be increased using plant suspension cell production in bioreactors, or by the propagation of stably transformed plant lines in the field. Transgenic plants can be exploited to produce organs rich in a recombinant protein for its long-term storage. This demonstrates the promise of using transgenic plants as bioreactors for the molecular farming of recombinant therapeutics, blood substitutes and diagnostics, without the expensive investment in infrastructure.

Vaccines

Another application that has been explored is the use of transgenic plants to produce vaccines (Haq et al. 1995). The binding subunit of *Escherichia coli* heat-labile enterotoxin (LT-B) is a highly active oral immunogen. Transgenic tobacco and potato plants were made with the use of genes encoding LT-B or an LT-B fusion protein with a microsomal retention sequence. The plants expressed the foreign peptides, both of which formed oligomers that bound the natural ligand. Mice immunized by gavage produced serum and gut mucosal anti-LT-B immunoglobulins that neutralized the enterotoxin in cell protection assays. Feeding mice fresh transgenic potato tubers also caused oral immunization.

Oral administration of protein can induce antigen-specific immune hyporesponsiveness. A transgenic plant was created which was capable of synthesizing the diabetes-associated autoantigen, glutamic acid decarboxylase (GAD). When administered as a dietary supplement, it inhibits the development of diabetes in the non-obese diabetic (NOD) mouse. In a similar fashion, a plant-derived edible vaccine against hepatitis B virus was developed (Ma et al. 1997). The DNA fragment encoding hepatitis B virus surface antigen was introduced into *Agrobacterium tumefaciens* LBA4404 and used to obtain transgenic lupin (*Lupinus luteus* L.) and lettuce (*Lactuca sativa* L.) cv. Burpee Bibb expressing envelope surface protein (Kapusta et al. 1999). Mice that were fed the transgenic lupin tissue developed significant levels of hepatitis B virus-specific antibodies. Human volunteers, fed with transgenic lettuce plants expressing hepatitis B virus surface antigen, developed specific serum-IgG response to plant produced protein.

A TMV-based vector has been used to express the complete open reading frame coding for VP1, the major immunogenic protein of foot and mouth disease virus (FMDV) in plants (Wigdorovitz et al. 1999). *In vitro* RNA transcripts were inoculated into *Nicotiana*

benthamiana plants and detectable amounts of recombinant VP1 were identified by Western blot as soon as 4 days post-infection. Foliar extracts prepared from infected leaves were injected intraperitoneally into mice and all of the immunized animals developed a specific antibody response to both the complete virus particle and the major immunogenic region as determined by ELISA and Western blot analysis. Most importantly, all immunized mice developed a protective immune response against experimental challenge with virulent FMDV. This is the first report showing the expression of a complete open reading frame of an antigenic foreign protein in plants (using a recombinant plant virus), in sufficient quantity to permit use of the crude plant extract as an experimental immunogen to protect animals against virus challenge.

Plant seeds offer unique opportunities for the production and delivery of oral subunit vaccines. The immunodominant glycoprotein B complex of human cytomegalovirus (HCMV), was introduced into tobacco plants, as a model system for studying the merit of this approach (Tackaberry et al. 1999). Given the advantages of expressing proteins in seeds, a novel expression vector was developed incorporating regulatory sequences of glutelin, the major rice seed storage protein, to direct synthesis of recombinant glycoprotein B. Analysis of genomic DNA of 28 selected tobacco transformants by PCR amplification showed that 71% harboured the gB cDNA. Specific immunoassays of protein extracts from seeds of positive plants showed that all were producing antigenic glycoprotein B at levels ranging from 70-146 ng/mg extracted protein. In addition, similarity with native glycoprotein B produced in HCMV-infected cells was also demonstrated by inhibition of immunofluorescence on HCMV-infected human fibroblasts. These data are the first to report the expression of an immunodominant antigen of HCMV in plant tissues, indicating the fidelity with which this very large heterologous viral glycoprotein can be synthesized in this model system.

The major structural protein VP60 of rabbit hemorrhagic disease virus (RHDV) has been produced in transgenic potato plants under the control of a cauliflower mosaic virus 35S promoter or a modified 35S promoter that included two copies of a strong transcriptional enhancer (Castanon et al. 1999). Both types of promoters allowed the production of specific mRNAs and detectable levels of recombinant VP60, which were higher for the constructs carrying the modified 35S promoter. Rabbits immunized with leaf extracts from plants carrying this modified 35S promoter showed high anti-VP60 antibody titers and were fully protected against the hemorrhagic disease.

Rapid production of protein-based tumor-specific vaccines for the treatment of malignancies is possible with the plant-based transient expression system (McCormick et al. 1999). A modified tobamoviral vector was made that encodes the idiotype-specific single-chain Fv fragment (scFv) of the immunoglobulin from the 38C13 mouse B cell lymphoma. Infected *Nicotiana benthamiana* plants contain high levels of secreted scFv protein in the extracellular compartment. This material reacts with an anti-idiotype antibody by Western blotting, ELISA, and affinity chromatography, suggesting that the plant-produced 38C13 scFv protein is properly folded in solution. Mice vaccinated with the affinity-purified 38C13 scFv generate >10 µg/ml anti-idiotype immunoglobulins. These mice were protected from challenge by a lethal dose of the syngeneic 38C13 tumor, similar to mice immunized with the native 38C13 IgM-keyhole limpet hemocyanin conjugate vaccine. This rapid production system for generating tumor-specific protein vaccines may provide a viable strategy for the treatment of non-Hodgkin's lymphoma.

Control of pandemic human immunodeficiency virus type 1 (HIV-1) infection ideally requires specific mucosal immunity to protect the genital regions through which transmission more often occurs. Thus a vaccine that stimulates a disseminated mucosal and systemic protective immune response would be extremely useful. A chimeric plant virus, cowpea mosaic virus (CPMV), expressing a 22 amino acid peptide (residues 731-752) of the transmembrane gp41 protein of HIV-1 IIIB (CPMV-HIV/1), was constructed. Stimulation of HIV-1-specific and CPMV-specific mucosal and serum antibody was assessed following intranasal or oral immunization together with the widely used mucosal adjuvant, cholera toxin. CPMV-HIV/1 has been shown to stimulate HIV-1-specific serum antibody in mice by parenteral immunization (Durrani et al. 1998). All mice immunized intranasally with two doses of 10 µg of CPMV-HIV/1 produced both HIV-1-specific IgA in faeces, as well as higher levels of specific, predominantly IgG2a, serum antibody. Additionally, there was a predominantly T helper 1 cell response. All mice also responded strongly to CPMV epitopes. Oral immunization of the chimeric cowpea mosaic virus was less effective, even at doses of 500 µg or greater, and stimulated HIV-1-specific serum antibody in only a minority of mice, and no faecal HIV-1-specific IgA.

Tomato plants were engineered (*Lycopersicon esculentum* Mill var. UC82b) to express a gene for the glycoprotein (G-protein), which coats the outer surface of the rabies virus (McGarvey et al. 1995). The recombinant constructs contained the G-protein gene from the ERA strain of rabies virus, including the signal peptide, under the control of the 35S promoter of cauliflower mosaic virus. Plants were transformed by *Agrobacterium tumefaciens*-mediated transformation of cotyledons and tissue culture on selective media. PCR confirmed the presence of the G-protein gene in plants surviving selection. Northern blot analysis indicated that RNA of the appropriate molecular weight was produced in both leaves and fruit of the transgenic plants. The recombinant G-protein was immunoprecipitated and detected by Western blot from leaves and fruit using different antisera. The G-protein expressed in tomato appeared as two distinct bands with apparent molecular mass of 62 and 60 kDa, as compared to the 66 kDa observed for G-protein from virus grown in BHK cells. Electron microscopy of leaf tissue using immunogold-labeling and antisera specific for rabies G-protein, showed localization of the G-protein to the Golgi bodies, vesicles, plasmalemma and cell walls of vascular parenchyma cells. In light of the fact that orally administered rabies G-protein from the same ERA strain elicits protective immunity in animals, these transgenic plants should provide a valuable tool for the development of edible oral vaccines.

Recombinant plant virus particles containing a chimeric peptide representing two rabies virus epitopes stimulate virus neutralizing antibody synthesis in immunized mice (Modelska et al. 1998). Mice immunized intraperitoneally or orally (by gastric intubation or by feeding on virus-infected spinach leaves) with engineered plant virus particles containing rabies antigen mount a local and systemic immune response. After the third dose of antigen, given intraperitoneally, 40% of the mice were protected against challenge infection with a lethal dose of rabies virus. Oral administration of the antigen stimulated serum IgG and IgA synthesis and ameliorated the clinical signs caused by intranasal infection with an attenuated rabies virus strain.

These studies demonstrate the ability of plants to manufacture authentic eucaryotic proteins, that in most cases, are properly folded and processed. Several vaccines have been shown to be capable of inducing protective immune responses, as demonstrated in animal models.

These potential vaccines are unique, in that they are ingested as food products and stimulate a mucosal immune response, which should also protect the lungs against air-borne challenges. As a genetic engineered food product, they may not require the same regulatory approval that a conventional vaccine requires.

Antibodies

Another example of the plants ability to correctly process mammalian proteins is the production of functional antibodies (Hiatt et al. 1989). Transgenic *Nicotiana tabacum* plants have been developed that express a murine monoclonal antibody kappa chain, a hybrid immunoglobulin A-G heavy chain, a murine joining chain, and a rabbit secretory component, respectively. Successive sexual crosses between these plants and filial recombinants resulted in plants that expressed all four protein chains simultaneously. These chains were assembled into a functional, high molecular weight secretory immunoglobulin that recognized the native streptococcal antigen, a cell surface adhesion molecule. In plants, single cells are able to assemble secretory antibodies, whereas two different cell types are required in mammals.

A functional recombinant TMV-specific full-size antibody (rAb29) was expressed in both the apoplast and cytosol of tobacco plants and a single chain antibody fragment (scFv29), derived from rAb29, was expressed in the cytosol (Schillberg et al. 1999). Cloned heavy and light chain cDNAs of full-size rAb29, which binds to TMV coat protein monomers, were integrated into the plant expression vector pSS. The full-size rAb29 was expressed in the cytosol and targeted to the apoplast by including the original murine antibody leader sequences. Levels of functional full-size rAb29 expression were high in the apoplast (up to 8.5 micrograms per gram leaf tissue), whereas cytosolic expression was low or, at the ELISA detection limit. Sequences of the variable domains of rAb29 light and heavy chain were used to generate the single chain antibody of scFv29, which was expressed in the periplasmic space of *E. coli* and showed the same binding specificity as full-size rAb29. In addition, scFv29 was functionally expressed in the cytosol of tobacco plants and plant derived scFv29 maintained the same binding specificity to TMV-coat protein monomers as rAb29.

The increasing use of monoclonal antibodies (mAbs) as diagnostic reagents necessitates efficient and cost-effective mAb production methods. In blood banks, one of the most routinely used reagents is the anti-human IgG reagent used for the detection of non-agglutinating antibodies. A functional, purified anti-human IgG (C5-1), was produced through the expression of its encoding genes in perennial transgenic alfalfa (Khoufi et al. 1999). Transgenic plants expressing the light- and heavy-chain cDNAs were obtained, and plants from crosses were found to express fully assembled C5-1. The purification procedure yielded mainly the bivalent form with specificity and affinity identical to those of hybridoma-derived C5-1. The ability to accumulate the antibody was maintained both in parental F1 lines during repeated harvesting and in clonal material; the antibody was stable in the drying hay as in extracts made in pure water. Also, plant and hybridoma-derived C5-1 had similar *in vivo* half-lives in mice. These results indicate that plant C5-1 could be used in a diagnostic reagent as effectively as hybridoma-derived C5-1, and demonstrates the usefulness of perennial systems for the cost-effective, stable, and reliable production of large amounts of mAbs.

Since plants are emerging as an important system for the expression of recombinant glycoproteins, especially those intended for therapeutic purposes, it is important to scrutinize to what extent glycans, harbored by mammalian glycoproteins and produced in transgenic plants, differ from their natural counterpart. The structures of the N-linked glycans attached to the heavy chains of the monoclonal antibody Guy's 13 produced in transgenic tobacco plants (plantibody Guy's 13) were identified and compared to those found in the corresponding IgG1 of murine origin (Cabanes-Macheteau et al. 1999). Both N-glycosylation sites located on the heavy chain of the plantibody Guy's 13 are N-glycosylated, as in mouse. However, the number of Guy's 13 glycoforms is higher in the plant than in the mammalian expression system. Despite the high structural diversity of the plantibody N-glycans, glycosylation appears to be sufficient for the production of a soluble and biologically active IgG in the plant system. In addition to high-mannose-type N-glycans, 60% of the oligosaccharides N-linked to the plantibody have $\beta(1, 2)$ -xylose and $\alpha(1, 3)$ -fucose residues linked to the core Man3GlcNAc2. These plant-specific oligosaccharide structures are not a limitation to the use of plantibody Guy's 13 for topical immunotherapy. However, their immunogenicity may raise concerns for systemic applications of plantibodies in humans.

The accumulation of five murine scFvs, binding to dihydroflavonol 4-reductase, was analyzed in transgenic *Petunia hybrida* plants (De Jaeger et al. 1999). The five scFv-encoding sequences were cloned in an optimized plant transformation vector for expression in the cytosol under control of the 35S promoter. In a transient expression assay, the scFv expression levels were reproducible and correlated with those in stably transformed petunia. The results show that accumulation in the cytosol strongly depends on the intrinsic properties of the scFv fragment. Three of the five scFv fragments accumulated to unexpectedly high levels in the cytosol of the primary transformants, but no phenotypic effect could be detected. Experimental results indicate that one of the scFv fragments accumulated in the cytosol, to 1% of the total soluble protein as a functional antigen-binding protein in the absence of disulphide bonds. This observation supports the idea that certain antibody fragments do not need disulphide bonds to be stable and functional. Such scFv scaffolds provide new opportunities to design scFv fragments for immunomodulation in the cytosol.

The ability to produce mAbs in plants offers the opportunity for the development of an inexpensive method of mucosal immunoprotection against sexually transmitted diseases. To investigate the suitability of plant-expressed mAbs for vaginal preventive applications, humanized anti-herpes simplex virus 2 (HSV-2) mAb was expressed in mammalian cell culture and in soybean (Zentlin et al. 1998). These mAbs were similar in their stability in human semen and cervical mucus over 24 h, in their ability to diffuse in human cervical mucus, and in their efficacy for prevention of vaginal HSV-2 infection in the mouse.

These studies point to the potential of plant expression of antibodies not only for the economical, large scale production of diagnostic reagents, but also for its administration as therapeutic biologicals for passive immunization. Plant cells also possess the requisite mechanisms for assembly and expression of other complex recombinant protein molecules, such as secretory IgA.

Other biologicals

The production of recombinant proteins for therapeutic use in transgenic plants has become an alternative to the isolation of these molecules from natural or other recombinant sources. Plant bioreactors offer the possibility of inexpensive, large scale production of significant volumes of recombinant proteins, combined with increased safety concerning contaminations with human pathogens. Several laboratories are currently involved in the commercial production of the first recombinant products, such as antibodies or recombinant human hemoglobin, from transgenic plants.

A plant oleosin was used as a 'carrier' for the production of the leech anticoagulant protein, hirudin (variant 2) (Parmenter et al. 1995). Seed-specific expression of the oleosin-hirudin fusion mRNA was directed via an *Arabidopsis* oleosin promoter. The fusion protein was correctly targeted to the oil body membrane and separated from the majority of other seed proteins by floatation centrifugation. Recombinant hirudin was localized to the surface of oil bodies, as determined by immunofluorescent techniques. The oleosin-hirudin fusion protein accumulated to ca. 1% of the total seed protein. Hirudin was released from the surface of the oil bodies using endoprotease treatment. The recombinant hirudin was partially purified through anion exchange and reverse-phase chromatography. Hirudin activity, measured in anti-thrombin units (ATU), was observed in seed oil body extracts, but only after the proteolytic release of hirudin from its oleosin 'carrier'. About 0.55 ATU per milligram of oil body protein was detected in cleaved oil body preparations. The oleosin fusion protein system provides a unique route for the large-scale production of recombinant proteins in plants, as well as an efficient process for purification of the desired polypeptide.

A modified CaMV 35S promoter was used to direct the expression of chimaeric genes encoding human serum albumin (HSA) in transgenic potato and tobacco plants (Sijmons et al. 1990). To secrete the protein, either the human prepro-sequence or the signal sequence from the extracellular tobacco protein PR-S was used. Secretion of HSA with both types of signal sequences in transgenic leaf tissue and in suspension cultures was observed. HSA produced in transgenic potato plants was purified to chromatographic homogeneity. N-terminal amino acid sequence analysis revealed that the processing of the precursor protein was dependent on the type of signal sequence. Expression of the human preproHSA gene led to partial processing of the precursor and secretion of proHSA. Fusion of HSA to the plant PR-S presequence resulted in cleavage of the presequence at its natural site and secretion of correctly processed HSA that is indistinguishable from the authentic human protein.

Tobacco plants were transformed with the human gene for interferon- β (IFN- β) (Edelbaum et al. 1992). Transformation efficiency was determined by PCR, and expression was determined by Western blot analysis, by purifying the IFN from the transgenic plants, and by bioassays indicating its activity in human cells. Plants expressing IFN- β were self-pollinated. IFN- β -expressing progeny plants were selected and produced active IFN- β , indicating stable transformation.

Erythropoietin (Epo), a glycoprotein that regulates the formation of erythrocytes in mammals, was produced in cultured tobacco BY2 cells (*Nicotiana tabacum* L. cv. Bright Yellow 2) by introducing human Epo cDNA via *Agrobacterium tumefaciens*-mediated gene transfer (Matsumoto et al. 1995). Epo was correctly processed and subsequently penetrated the

plasma membrane of tobacco cells. However, it remained attached to the cell wall and was not released into the culture medium. Although Epo produced by tobacco cells was glycosylated with N-linked oligosaccharides, these carbohydrates were smaller than those of the recombinant Epo produced in mammalian cells. Epo produced in tobacco exhibited *in vitro* biological activities by inducing the differentiation and proliferation of erythroid cells. However, it retained no *in vivo* biological activities. A lectin-binding assay indicated the lack of sialic acid residues in the N-linked oligosaccharides of Epo, suggesting that Epo was removed from the circulation before it reached erythropoietic tissues, and is one of the few examples of a plant product not exhibiting the desired properties.

Human growth hormone (hGH) gene has been inserted into the plasmid pLGV1103 to give the recombinant plasmid pLB-9 (Li et al. 1994). It has been introduced into the agrobacterium containing plasmid pGV3850, and the recombinant Ti plasmid pGL198(hGH) obtained by homologous recombination. The monocotyledon *Caladium bicolor* has been transformed with pGL198 (hGH) with the leaf-disk co-cultivation method, and transgenic plants have been regenerated. The results of nopaline analysis, npt II detection, Southern blot and Western blot, showed that the hGH gene was integrated into the genome of *Caladium bicolor*, and a 22-kD protein was synthesized.

Recently, various human blood coagulation factors, including Factor VIII and Factor IX were expressed in plants (Hooker et al. 1999). The plant-derived products were capable of eliciting an activation in human blood clotting pathways and provide a virus-free product for the treatment of humans diagnosed with blood diseases. This represents additional proof of the utility of plant expression, since these factors are often quite large and require extensive post-translational modifications such as glycosylation, γ -carboxylation of specific glutamic acid residues, and β -hydroxylation of aspartic acid residues.

Conclusion

Plants represent a fascinating alternative eukaryotic expression system for the production of xenogenic products, as is evident from the numerous examples presented in this review. A wide variety of proteins have been successfully expressed in plants, ranging from vaccines to antibodies to biologicals. Bacteria are highly efficient expression systems, however, eucaryotic proteins are not always processed correctly to an active form, and post-translational processing may be absent (glycosylation). Eukaryotic processing has not been an obstacle, to date, to producing complex heterologous proteins with specific activities equivalent to that obtained with other systems. Differences in plant vs mammalian glycosylation could affect the activity of the expressed protein, however, in most instances, any observed variation did not affect the biological activity.

Plants are an economical system for the production of a wide variety of molecules including products that are highly toxic to other systems (Appendix A). Relative to the cost of fermentation, plant genetic engineering of biologicals (estimated to be 10 to 50 fold lower in cost than *E. coli* expression) potentially represent an inexpensive, albeit uncharacterized, method of producing biological toxins.

In summary, genetic engineering of plants represents an exciting, alternative system for the production of vaccines and antibodies for protection against microorganisms. Limitations in the ability to produce scarce proteins must be re-evaluated in light of the advances in plant biotechnology.

References

1. Anderson, J.M., P. Palukartis, and M. Zaitlin. 1992. A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA* 89:8759-8763.
2. Angell, S.M., and D.C. Baulcombe. 1997. Consistent gene silencing in transgenic plants expressing a replicating potato virus X RNA. *EMBO J.* 16:3675-3684.
3. Angenent, G.C., J.M.W. van Den Ouweland, and J.F. Bol. 1990. Susceptibility to virus infection of transgenic tobacco plants expressing structural and nonstructural genes of tobacco rattle virus. *Virology* 175:191-198.
4. Ares, X., G. Calamante, S. Cabral, J. Lodge, P. Hemenway, R.N. Beachy, and A. Mentaberry. 1998. Transgenic plants expressing potato virus X ORF2 protein (p24) are resistant to tobacco mosaic virus and Ob tobamoviruses. *J. Virol.* 72:731-738.
5. Audy, P., P. Palukartis, S.A. Slack, and M. Zaitlin. 1994. Replicase-mediated resistance to potato virus Y in transgenic tobacco plants. *Mol. Plant-Microbe Interact.* 7:15-22.
6. Bardonnet, N., F. Hans, M.A. Serghini, and L. Pinck. 1994. Protection against virus infection in tobacco plants expressing the coat protein of grapevine fanleaf nepovirus. *Plant Cell Rep.* 13:357-360.
7. Barker, H., B. Reavy, A. Kumar, K.D. Webster, and M.A. Mayo. 1992. Restricted virus multiplication in potatoes transformed with the coat protein gene of potato leafroll luteovirus: similarities with a type of host gene-mediated resistance. *Ann. Appl. Biol.* 120:55-64.
8. Barker, H., B. Reavy, K.D. Webster, C.A. Jolly, A. Kumar, and M.A. Mayo. 1993. Relationship between transcript production and virus resistance in transgenic tobacco expressing the potato leafroll virus coat protein gene. *Plant Cell Rep.* 13:54-58.
9. Barker, H., K.D. Webster, C.A. Jolly, B. Reavy, A. Kumar, and M.A. Mayo. 1994. Enhancement of resistance to potato leafroll virus multiplication in potato by combining the effects of host genes and transgenes. *Mol. Plant-Microbe Interact.* 7:528-530.
10. Baulcombe, D.C. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. *Plant Cell* 8:1833-1844.
11. Beachy, R.N., S. Loesch-Fries, and N.E. Turner. 1990. Coat protein-mediated resistance against virus infection. *Annu. Rev. Phytopathol.* 28:451-474.
12. Bendahmane, M., and B. Gronenborn. 1997. Engineering resistance against tomato yellow leaf curl virus (TYLCV) using antisense RNA. *Plant Mol. Biol.* 33:351-357.

13. Bendahmane, M., J.H. Fitch, G. Zhang, and R.N. Beachy. 1997. Studies of coat protein-mediated resistance to tobacco mosaic tobamovirus: correlation between assembly of mutant coat proteins and resistance. *J. Virol.* 71:7942-7950.
14. Beck, D.L., C.J. van Dolleweerd, T.J. Lough, E. Balmori, D.M. Voot, M.T. Andersen, I.E.W. O'Brien, and R.L.S. Forster. 1994. Disruption of virus movement confers broad-spectrum resistance against systemic infection by plant viruses with a triple gene block. *Proc. Natl. Acad. Sci. USA* 91:10310-10314.
15. Bertoli, D.J., J.I. Cooper, M.L. Edwards, and W.S. Hawes. 1992. Arabis mosaic nepovirus coat protein in transgenic tobacco lessens disease severity and virus replication. *Ann. Appl. Biol.* 120:47-54.
16. Brault, V., T. Candresse, O. le Gall, R.P. Delbos, M. Lanneau, and J. Dunez. 1993. Genetically engineered resistance against grapevine chrome mosaic nepovirus. *Plant Mol. Biol.* 21:89-97.
17. Braun, C.J., and C.L. Hemenway. 1992. Expression of amino-terminal portions or full-length viral replicase genes in transgenic plants confers resistance to potato virus X infection. *Plant Cell* 4:735-744.
18. Brederode, F.T., P.E.M. Taschner, E. Posthumus, and J.F. Bol. 1995. Replicase-mediated resistance to alfalfa mosaic virus. *Virology* 207:467-474.
19. Cabanes-Macheteau, M., A.C. Fitchette-Laine, C. Loutelier-Bourhis, C. Lange, N.D. Vine, J.K. Ma, P. Lerouge, and L. Faye. 1999. N-Glycosylation of a mouse IgG expressed in transgenic tobacco plants. *Glycobiology* 9:365-372.
20. Carr, J.P., and M. Zaitlin. 1991. Resistance in transgenic tobacco plants expressing a nonstructural gene sequence of tobacco mosaic virus is a consequence of markedly reduced virus replication. *Mol. Plant-Microbe Interact.* 4:579-585.
21. Carr, J.P., A. Gal-On, P. Palukaitis, and M. Zaitlin. 1994. Replicase-mediated resistance to cucumber mosaic virus in transgenic plants involves suppression of both virus replication in the inoculated leaves and long-distance movement. *Virology* 199:439-447.
22. Cassidy, B.G., and R.S. Nelson. 1995. Differences in protection phenotypes in tobacco plants expressing coat protein genes from peanut stripe potyvirus with or without an engineered ATG. *Mol. Plant-Microbe Interact.* 8:357-365.
23. Castanon, S., M.S. Marin, J.M. Martin-Alonso, J.A. Boga, R. Casais, J.M. Humara, R.J. Ordas, and F. Parra. 1999. Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. *J. Virol.* 73: 4452-4455.
24. Chia, T.-F., Y.-S. Chan, and N.-H. Chua. 1992. Characterization of cymbidium mosaic virus coat protein gene and its expression in transgenic tobacco plants. *Plant Mol. Biol.* 18:1091-1099.

25. Clark, W.G., J.H. Fitchen, and R.N. Beachy. 1995a. Studies of coat protein-mediated resistance to TMV. I. The PM2 assembly defective mutant confers resistance to TMV. *Virology* 208:485-491.
26. Clark, W.G., J. Fitchen, A. Nejdat, C.M. Deom, and R.N. Beachy. 1995b. Studies of coat protein-mediated resistance to tobacco mosaic virus (TMV). II. Challenge by a mutant with altered virion surface does not overcome resistance conferred by TMV coat protein. *J. Gen. Virol.* 76:2613-2617.
27. Collmer, C.W., and S.H. Howell. 1992. Role of satellite RNA in the expression of symptoms caused by plant viruses. *Annu. Rev. Phytopathol.* 30: 419-442.
28. Cooper, B., M. Lapidot, J.A. Heick, J.A. Dodds, and R.N. Beachy. 1995. A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility. *Virology* 206:307-313.
29. Cuzzo, M., K.M. O'Connell, W. Kaniewski, R.-X. Fang, N.-H. Chua, and N.E. Turner. 1988. Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. *Bio/Technology* 6:549-557.
30. Day, A.G., E.R. Bejarano, K.W. Buck, M. Burrell, and C.P. Lichtenstein. 1991. Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus. *Proc. Natl. Acad. Sci. USA* 88:6721-6725.
31. de Feyter, R., M. Young, K. Schroeder, E.S. Dennis, and W. Gerlach. 1996. A ribozyme gene and an antisense gene are equally effective in conferring resistance to tobacco mosaic virus on transgenic tobacco. *Mol. Gen. Genet.* 250:329-338.
32. de Haan, P., J.J.L. Gielen, M. Prins, I.G. Wijkamp, A. van Schepen, D. Peters, M.Q.J.M. van Grinsven, and R. Goldbach. 1992. Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants. *Bio/Technology* 10:1133-1137.
33. De Jaeger, G., E. Buys, D. Eeckhout, C. De Wilde, A. Jacobs, J. Kapila, G. Angenon, M. Van Montagu, T. Gerats, and A. Depicker. 1999. High level accumulation of single-chain variable fragments in the cytosol of transgenic *Petunia hybrida*. *Eur. J. Biochem.* 259:426-434.
34. Deom, C.M., M. Lapidot, and R.N. Beachy. 1992. Plant virus movement proteins. *Cell* 69:221-224.
35. Derrick, P.M., and H. Barker. 1997. Short and long distance spread of potato leafroll luteovirus: effects of host genes and transgenes conferring resistance to virus accumulation in potato. *J. Gen. Virol.* 78:243-251.
36. Dinant, S., F. Blaise, C. Kusiak, S. Astier-Manificier, and J. Albouy. 1993. Heterologous resistance to potato virus Y in transgenic tobacco plants expressing the coat protein gene of lettuce mosaic potyvirus. *Phytopathology* 83:818-824.

37. Dinesh-Kumar, S.P., S. Whitham, D. Choi, R. Hehl, C. Corr, and B. Baker. 1995. Transposon tagging of tobacco mosaic virus resistance gene N: Its possible role in the TMV-N-mediated signal transduction pathway. *Proc. Natl. Acad. Sci. USA* 92:4175-4180.
38. Donson, J., C.M. Kearney, T.H. Turpen, I.A. Khan, G. Kurath, A.M. Turpen, G.E. Jones, W.O. Dawson, and D.J. Lewandowski. 1993. Broad resistance to tobamoviruses is mediated by a modified tobacco mosaic virus replicase transgene. *Mol. Plant-Microbe Interact.* 6:635-642.
39. Dougherty, W.G., and J.C. Carrington. 1988. Expression and function of potyviral gene products. *Annu. Rev. Phytopathol.* 26:123-143.
40. Durrani, Z., T.L. McInerney, L. McLain, T. Jones, T. Bellaby, F.R. Brennan, and N.J. Dimmock. 1998. Intranasal immunization with a plant virus expressing a peptide from HIV-1 gp41 stimulates better mucosal and systemic HIV-1-specific IgA and IgG than oral immunization. *J. Immunol. Methods* 220:93-103.
41. Edelbaum, O., D. Stein, N. Holland, Y. Gafni, O. Livneh, D. Novick, M. Rubinstein, and I. Sela. 1992. Expression of active human interferon-beta in transgenic plants. *J. Interferon Res.* 12: 449-453.
42. English, J.J., E. Mueller, and D.C. Baulcombe. 1996. Suppression of virus accumulation in transgenic plants exhibiting silencing of nuclear genes. *Plant Cell* 8:179-188.
43. Ewen, S., and Pusztai, A. 1999. Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 354:1353-1354.
44. Fang, G., and R. Grunet. 1993. Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. *Mol. Plant-Microbe Interact.* 6:358-367.
45. Fecker, L.F., R. Koenig, and C. Obermeier. 1997. *Nicotiana benthamiana* plants expressing beet necrotic yellow vein virus (BNYVV) coat protein-specific scFv are partially protected against the establishment of the virus in the early stages of infection and its pathogenic effects in the late stages of infection. *Arch. Virol.* 142:1857-1863.
46. Fitch, M.M.M., R.M. Manshardt, D. Gonsalves, J.L. Slightom, and J.C. Sanford. 1992. Virus resistant papaya plants derived from tissues bombarded with the coat protein gene of papaya ringspot virus. *Bio/Technology* 10:1466-1472.
47. Fitchen, J.H., and R.N. Beachy. 1993. Genetically engineered protection against viruses in transgenic plants. *Annu. Rev. Microbiol.* 47:739-763.
48. Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann, and S.C. Woo. 1983. Expression of bacterial genes in plant cells. *Proc. Natl. Acad. Sci. USA* 80:4803-4807.

49. Fraley, R.T., S.G. Rogers, and R.B. Horsch. 1985. Genetic transformation in higher plants. In "CRC Critical Reviews in Plant Sciences. Vol. 4: 1-46.
50. Frischmuth, T., and J. Stanley. 1994. Beet curly top virus symptom amelioration in *Nicotiana benthamiana* transformed with a naturally occurring viral subgenomic DNA. *Virology* 200:826-830.
51. Fuchs, M., and D. Gonsalves. 1995. Resistance of transgenic hybrid squash ZW-20 expressing the coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses. *Bio/Technology* 13:1466-1473.
52. Gerlach, W.L., D. Llewellyn, and J. Haseloff. 1987. Construction of a plant disease resistance gene from the satellite RNA of tobacco ringspot virus. *Nature* 328:802-805.
53. Gielen, J.J.L., P. de Haan, A.J. Kool, D. Peters, M.Q.J.M. van Grinsven, and R.W. Goldbach. 1991. Engineered resistance to tomato spotted wilt virus, a negative-strand RNA virus. *Bio/Technology* 9:1363-1367.
54. Golemboski, D.B., G.P. Lomonossoff, and M. Zastlin. 1990. Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus. *Proc. Natl. Acad. Sci. USA* 87:6311-6315.
55. Gonsalves, D., P. Chee, R. Provvidenti, R. Seem, and J.L. Slightom. 1992. Comparison of coatprotein-mediated and genetically-derived resistance in cucumbers to infection by cucumber mosaic virus under field conditions with natural challenge inoculations by vectors. *Bio/Technology* 10:1562-1570.
56. Goodwin, J., K. Chapman, S. Swaney, T.D. Parks, E.A. Wernsman, and W.G. Dougherty. 1996. Genetic and biochemical dissection of transgenic RNA-mediated virus resistance. *Plant Cell* 8:95-105.
57. Graham, M.W., S. Craig, and P.M. Waterhouse. 1997. Expression patterns of vascular-specific promoters RolC and Sh in transgenic potatoes and their use in engineering PLRV-resistant plants. *Plant Mol. Biol.* 33:729-735.
58. Grumet, R. 1995. Genetic engineering for crop virus resistance. *HortScience* 30:449-456.
59. Guo, H.S., M.T. Cervera, and J.A. Garcia. 1998. Plum pox potyvirus resistance associated to transgene silencing that can be stabilized after different number of plant generations. *Gene* 206:263-272.
60. Hackland, A.F., E.P. Rybicki, and J.A. Thomson. 1994. Coat protein-mediated resistance in transgenic plants. *Arch. Virol.* 139:1-22.
61. Hamilton, R.I. 1980. Defenses triggered by previous invaders: viruses. In: *Plant Disease: An Advanced Treatise*, Vol. 5. J.G. Horsfall and E.B. Cowling, eds., pp. 279-303. Academic Press, New York.

62. Hammond, J., and K.K. Kamo. 1993. Effective resistance to potyvirus infection conferred by expression of antisense RNA in transgenic plants. *Mol. Plant Microbe Interact.* 8:674-682.
63. Hammond, J., and M.M. Dienelt. 1997. Encapsidation of potyviral RNA in various forms of transgene coat protein is not correlated with resistance in transgenic plants. *Mol. Plant-Microbe Interact.* 10:1023-1027.
64. Haq, T.A., H.S. Mason, J.D. Clements, and C.J. Arntzen. 1995. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. *Science* 268: 714-716.
65. Harrison, B.D., M.A. Mayo, and D.C. Baulcombe. 1987. Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. *Nature* 328:799-802.
66. Hayakawa, T., Y. Zhu, K. Itoh, Y. Kimura, T. Izawa, K. Shimamoto, and S. Toriyama. 1992. Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus. *Proc. Natl. Acad. Sci. USA* 89:9865-9869.
67. Hellwald, K.-H., and P. Palukaitis. 1995. Viral RNA as a potential target for two independent mechanisms of replicase-mediated resistance against cucumber mosaic virus. *Cell* 83:937-946.
68. Hemenway, C., R.-X. Fang, W.K. Kaniewski, N.-H. Chua, and N.E. Turner. 1988. Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. *EMBO J.* 7:1273-1280.
69. Hiatt, A., R. Cafferkey, and K. Bowdish. 1989. Production of antibodies in plants. *Nature* 342: 76- 78.
70. Hill, K.K., N. Jarvis-Eagan, E.L. Halk, K.J. Krahn, L.W. Liao, R.S. Mathewson, D.J. Merlo, S.E. Nelson, K.E. Rashka, and L.S. Loesch-Fries. 1991. The development of virus-resistant alfalfa *Medicago sativa* L. *Bio/Technology* 9:373-377.
71. Hillman, B.I., J.C. Carrington, and T.J. Morris. 1987. A defective interfering RNA that contains a mosaic of a plant virus genome. *Cell* 51:427-433.
72. Hoekema, A., M.J. Huisman, L. Molendijk, P.J.M. van den Elzen, and B.J.C. Cornelissen. 1989. The genetic engineering of two commercial potato cultivars for resistance to potato virus X. *Bio/Technology* 7:273-278.
73. Hong, Y., and J. Stanley. 1996. Virus resistance in *Nicotiana benthamiana* conferred by African cassava mosaic virus replication-associated protein (AC1) transgene. *Mol. Plant-Microbe Interact.* 9:219-225.
74. Hong, Y., K. Saunders, M.R. Hartley, and J. Stanley. 1996. Resistance to geminivirus infection by virus-induced expression of diathin in transgenic plants. *Virology* 220:119-127.

75. Hooker, B.S., J. Gao, D.B. Anderson, and Z. Dai. 1999. Transgenic plant-derived human blood coagulation factors. WO9958699.
76. Horsch, R.B., J.E. Fry, N.L. Hoffmann, D. Eichholtz, S.G. Rogers, and R.T. Fraley. 1985. A simple and general method for transferring genes into plants. *Science* 227:1229-1231.
77. Huang, F., L.L. Buschman, R.A. Higgins, and W.H. McGaughey. 1999. Inheritance of Resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science* 284: 965-967.
78. Hull, R., and J.W. Davies. 1992. Approaches to nonconventional control of plant virus diseases. *Crit. Rev. Plant Sci.* 11:17-33.
79. Jacquemond, M., J. Amselem, and M. Tepfer. 1988. A gene coding for a monomeric form of cucumber mosaic virus satellite RNA confers tolerance to CMV. *Mol. Plant-Microbe Interact.* 1:311-316.
80. Kaniewski, W., C. Lawson, B. Sammons, L. Haley, J. Hart, X. Delannay, and N.E. Turner. 1990. Field resistance of transgenic Russet Burbank potato to effects of infection by potato virus X and potato virus Y. *Bio/Technology* 8:750-754.
81. Kapusta, J., A. Modelska, M. Figlerowicz, T. Pniewski, M. Letellier, O. Lisowa, V. Yusibov, H. Koprowski, A. Plucieniczak, and A.B. Legocki. 1999. A plant-derived edible vaccine against hepatitis B virus. *FASEB J.* 13:1796-1799.
82. Kawchuk, L.M., R.R. Martin, and J. McPherson. 1990. Resistance in transgenic potato expressing the potato leafroll virus coat protein gene. *Mol. Plant-Microbe Interact.* 3: 301-307.
83. Kawchuk, L.M., R.R. Martin, and J. McPherson. 1991. Sense and antisense RNA-mediated resistance to potato leafroll virus in Russet Burbank potato plants. *Mol. Plant-Microbe Interact.* 4:247-253.
84. Kawchuk, L.M., D.R. Lynch, R.R. Martin, G.C. Kozub, and B. Farries. 1997. Field resistance to the potato leafroll luteovirus reduces tuber disease symptoms in transgenic and somaclonal variant potato plants. *Can. J. Plant Pathol.* 19: 260-266.
85. Khoudi, H., S. Laberge, J.M. Ferullo, R. Bazin, A. Darveau, Y. Castonguay, G. Allard, R. Lemieux, and L.P. Vezina. 1999. Production of a diagnostic monoclonal antibody in perennial alfalfa plants. *Biotechnol. Bioeng.* 64:135-143.
86. Kollar, A., T. Dalmay, and J. Burgyan. 1993. Defective interfering RNA-mediated resistance against cymbidium ringspot tobravirus in transgenic plants. *Virology* 193:313-318.

87. Kunik, T., R. Salomon, D. Zamur, N. Navot, M. Zeidan, I. Michelson, Y. Gafni, and H. Czosnek. 1994. Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. *Bio/Technology* 12:500-504.
88. Kwon, C.S., W.I. Chung, and K.H. Paek. 1997. Ribozyme mediated targeting of cucumber mosaic virus RNA 1 and 2 in transgenic tobacco plants. *Mol. Cells* 7:326-334.
89. Lapidot, M., R. Gafny, B. Ding, S. Wolf, W.J. Lucas, and R.N. Beachy. 1993. A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants. *Plant J.* 4:959-970.
90. Lawson, C., W. Kaniewski, L. Haley, R. Rozman, C. Newell, P. Sanders, and N.E. Tumer. 1990. Engineering resistance to mixed virus infection in a commercial potato cultivar: resistance to potato virus X and potato virus Y in transgenic Russet Burbank. *Bio/Technology* 8:127-134.
91. Leclerc, D., and M.G. AbouHaidar. 1995. Transgenic tobacco plants expressing a truncated form of the PAMV capsid protein (CP) gene show CP-mediated resistance to potato aucuba mosaic virus. *Mol. Plant-Microbe Interact.* 8:58-65.
92. Li, B.J., J.F. Wang, Z.F. Xu, Y.Q. Xu, M.Z. Yu, X. He, and Y. Shen. 1994. Integration and expression of human growth hormone gene in *Caladium bicolor*. *Sci. China B.* 37:280-285.
93. Lim, P.O., J.S. Ryu, H.J. Lee, U. Lee, Y.S. Park, J.M. Kwak, J.K. Choi, and H.G. Nam. 1997. Resistance to tobamoviruses in transgenic tobacco plants expressing the coat protein gene of pepper mild mottle virus (Korean isolate). *Mol. Cells* 7:313-319.
94. Lindbo, J.A., and W.G. Dougherty. 1992. Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. *Virology* 189:725-733.
95. Lindbo, J.A., L. Silva-Rosales, W.M. Proebsting, and W.G. Dougherty. 1993. Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. *Plant Cell* 5:1749-1759.
96. Ling, K., S. Namba, C. Gonsalves, J.L. Slightom, and D. Gonsalves. 1991. Protection against detrimental effects of potyvirus infection in transgenic tobacco plants expressing the papaya ringspot virus coat protein gene. *Bio/Technology* 9:752-758.
97. Lodge, J.K., W.K. Kaniewski, and N.E. Tumer. 1993. Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. *Proc. Natl. Acad. Sci. USA* 90:7089-7093.
98. Loesch-Fries, L.S., D. Merlo, D., T. Zinnen, L. Burhop, K. Hill, K. Krahn, N. Jarvis, S. Nelson, and E. Halk. 1987. Expression of alfalfa mosaic virus RNA 4 in transgenic plants confers virus resistance. *EMBO J.* 6:1845-1851.

99. Longstaff, M., G. Brignetti, F. Boccard, S. Chapman, and D. Baulcombe. 1993. Extreme resistance to potato virus X infection in plants expressing a modified component of the putative viral replicase. *EMBO J.* 12:379-386.
100. Ma, S.W., D.L. Zhao, Z.Q. Yin, R. Mukherjee, B. Singh, H.Y. Qin, C.R. Stiller, and A.M. Jevnikar. 1997. Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nat. Med.* 3:793-796.
101. MacFarlane, S.A., and J.W. Davies. 1992. Plants transformed with a region of the 201-kilodalton replicase gene from pea early browning virus RNA1 are resistant to virus infection. *Proc. Natl. Acad. Sci. USA* 89:5829-5833.
102. MacKenzie, D.J., and J.H. Tremaine. 1990. Transgenic *Nicotiana debneyi* expressing viral coat protein are resistant to potato virus S infection. *J. Gen. Virol.* 71: 2167-2170.
103. MacKenzie, D.J., and P.J. Ellis. 1992. Resistance to tomato spotted wilt virus infection in transgenic tobacco expressing the viral nucleocapsid gene. *Mol. Plant-Microbe Interact.* 5:34-40.
104. MacKenzie, D.J., J.H. Tremaine, and J. McPherson. 1991. Genetically engineered resistance to potato virus S in potato cultivar Russet Burbank. *Mol. Plant-Microbe Interact.* 4:95-102.
105. Maiti, I.B., J.F. Murphy, J.G. Shaw, and A.G. Hunt. 1993. Plants that express a potyvirus proteinase gene are resistant to virus infection. *Proc. Natl. Acad. Sci. USA* 90:6110-6114.
106. Malysenko, S.I., O.A. Kondakova, J.V. Nazarova, I.B. Kaplan, M.E. Talianky, and J.G. Atabekov. 1993. Reduction of tobacco mosaic virus accumulation in transgenic plants producing non-functional viral transport proteins. *J. Gen. Virol.* 74:1149-1156.
107. Marcos, J.F., and R.N. Beachy. 1997. Transgenic accumulation of two plant virus coat proteins on a single self-processing polypeptide. *J. Gen. Virol.* 78:1771-1778.
108. McCormick, A.A., M.H. Kumagai, K. Hanley, T.H. Turpen, I. Hakim, L.K. Grill, D. Tuse, S. Levy, and R. Levy. 1999. Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. *Proc. Natl. Acad. Sci. U S A* 96:703-708.
109. Matsumoto, S., K. Ikura, M. Ueda, and R. Sasaki. 1995. Characterization of a human glycoprotein (erythropoietin) produced in cultured tobacco cells. *Plant Mol. Biol.* 27:1163-1172.
110. McGarvey, P.B., J. Hammond, M.M. Dienelt, D.C. Hooper, Z.F. Fu, B. Dietzschold, H. Koprowski, and F.H. Michaels. 1995. Expression of the rabies virus glycoprotein in transgenic tomatoes. *Biotechnology* 13:1484-1487.

111. McKinney, H.H. 1929. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. *J. Agric. Res.* 39: 557-578.
112. Mellor, F.C., and R. Stace-Smith. 1977. Virus-free potatoes by tissue culture. In *Applied and Fundamental Aspects of Plant Cell, Tissue Culture, and Organ Culture*. J. Reinert and Y.P.S. Bajaj, Eds. Springer-Verlag, Berlin, Heidelberg New York. Pp 616-635.
113. Modelska, A., B. Dietzschold, N. Sleysh, Z.F. Fu, K. Steplewski, D.C. Hooper, H. Koprowski, and V. Yusbov. 1998. Immunization against rabies with plant-derived antigen. *Proc. Natl. Acad. Sci. U S A* 95:2481-2485.
114. Moon, Y.H., S.K. Song, K.W. Choi, and J.S. Lee. 1997. Expression of a cDNA encoding *Phytolacca insularis* antiviral protein confers virus resistance on transgenic potato plants. *Mol. Cells* 7:807-815.
115. Mueller, E., J. Gilbert, G. Davenport, G. Brigneti, and D.C. Baulcombe. 1995. Homology- dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. *Plant J.* 7:1001-1013.
116. Murry, L.E., L.G. Elliott, S.A. Capitani, J.A. West, K.K. Hanson, L. Scarafia, S. Johnston, C. DeLuca-Flaherty, S. Nichols, D. Cunanan, P.S. Dietrich, L.J. Mettler, S. Dewald, D.A. Warnick, C. Rhodes, R.M. Simibaldi, and K.J. Brunke. 1993. Transgenic corn plants expressing MDMV strain B coat protein are resistant to mixed infections of maize dwarf mosaic virus and maize chlorotic mottle virus. *Bio/Technology* 11:1559-1564.
117. Nakajima, M., T. Hayakawa, I. Nakamura, and M. Suzuki. 1993. Protection against cucumber mosaic virus (CMV) strains O and Y and chrysanthemum mild mottle virus in transgenic tobacco plants expressing CMV-O coat protein. *J. Gen. Virol.* 74:319-322.
118. Namba, S., K. Ling, C. Gonsalves, D. Gonsalves, and J.L. Slightom. 1991. Expression of the gene encoding the coat protein of cucumber mosaic virus (CMV) strain WL appears to provide protection to tobacco plants against infection by several different CMV strains. *Gene* 107:181-188.
119. Namba, S., K. Ling, C. Gonsalves, J.L. Slightom, and D. Gonsalves. 1992. Protection of transgenic plants expressing the coat protein gene of watermelon mosaic virus II or zucchini yellow mosaic virus against six potyviruses. *Phytopathology* 82:940-946.
120. Nejdat, A., and R.N. Beachy. 1989. Decreased levels of TMV coat protein in transgenic tobacco plants at elevated temperatures reduce resistance to TMV infection. *Virology* 173:531-538.
121. Nelson, A., D.A. Roth, and J.D. Johnson. 1993. Tobacco mosaic virus infection of transgenic *Nicotiana tabacum* plants is inhibited by antisense constructs directed at the 5' region of viral RNA. *Gene* 127:227-232.

122. Nelson, R.S., S.M. McCormick, X. Delannay, P. Dube, J. Layton, E.J. Anderson, M. Kaniewska, R.K. Proksch, R.B. Horsch, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1988. Virus tolerance, plant growth, and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. *Bio/Technology* 6:403-409.
123. Nguyen, L., W.J. Lucas, B. Ding, and M. Zaitlin. 1996. Viral RNA trafficking is inhibited in replicase-mediated resistant transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA* 93:12643-12647.
124. Noris, E., G.P. Accotto, R. Tavazza, A. Brunetti, S. Crespi, and M. Tavazza. 1996. Resistance to tomato yellow leaf curl geminivirus in *Nicotiana benthamiana* plants transformed with a truncated viral C1 gene. *Virology* 224:130-138.
125. Palucha, A., W. Zagorski, M. Chrzanowska, and D. Hulanicka. 1997. An antisense coat protein gene confers immunity to potato leafroll virus in genetically-engineered potato. *Eur. J. Plant Pathol.* 104:287-293.
126. Palukaitis, P., and M. Zaitlin. 1997. Replicase-mediated resistance to plant virus disease. *Adv. Virus Res.* 48:349-377.
127. Pang, S.-Z., J.L. Slightom, and D. Gonsalves. 1993. Different mechanisms protect transgenic tobacco against tomato spotted wilt and impatiens necrotic spot tospoviruses. *Bio/Technology* 11:819-824.
128. Pang, S.-Z., F.-J. Jan, and D. Gonsalves. 1997. Nontarget DNA sequences reduce the transgene length necessary for RNA-mediated tospovirus resistance in transgenic plants. *Proc. Natl. Acad. Sci. USA* 94:8261-8266.
129. Pang, S.-Z., P. Nagpala, M. Wang, J.L. Slightom, and D. Gonsalves. 1992. Resistance to heterologous isolates of tomato spotted wilt virus in transgenic tobacco expressing its nucleocapsid protein gene. *Phytopathology* 82:1223-1229.
130. Pang, S.-Z., J.H. Bock, C. Gonsalves, J.L. Slightom, and D. Gonsalves. 1994. Resistance of transgenic *Nicotiana benthamiana* plants to tomato spotted wilt and impatiens necrotic spot tospoviruses: evidence of involvement of the N protein and N gene RNA in resistance. *Phytopathology* 84:243-249.
131. Pang, S.-Z., F.-J. Jan, K. Carney, J. Stout, D.M. Tricoli, H.D. Quemada, and D. Gonsalves. 1996. Post-transcriptional transgene silencing and consequent tospovirus resistance in transgenic lettuce are affected by transgene dosage and plant development. *Plant J.* 9:899-909.
132. Pappu, H.R., C.L. Niblett, and R.F. Lee. 1995. Application of recombinant DNA technology to plant protection: molecular approaches to engineering virus resistance in crop plants. *World J. Microbiol. & Biotech.* 11:426-437.

133. Parmenter, D.L., J.G. Boothe, G.J. van Rooijen, E.C. Yeung, and M.M. Moloney. 1995. Production of biologically active hirudin in plant seeds using oleosin partitioning. *Plant Mol. Biol.* 29:1167-1180.
134. Poirer, Y., D.E. Dennis, K. Klomparens, and C. Sommerville. 1992. Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants. *Science* 256: 520-523.
135. Powell, P.A., D.M. Stark, P.R. Sanders, and R.N. Beachy. 1989. Protection against tobacco mosaic virus in transgenic plants that express tobacco mosaic virus antisense RNA. *Proc. Natl. Acad. Sci. USA* 86:6949-6952.
136. Powell, P.A., P.R. Sanders, N. Turner, R.T. Fraley, and, R.N. Beachy. 1990. Protection against tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences. *Virology* 175:124-130.
137. Powell, P.A., R.S. Nelson, B. De, N. Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232:738-743.
138. Presting, G.G., O.P. Smith, and C.R. Brown. 1995. Resistance to potato leafroll virus in potato plants transformed with the coat protein gene or with vector control constructs. *Phytopathology* 85:436-442.
139. Prins, M., P. de Haan, R. Luyten, M. van Veller, M.Q.J.M. van Grinsven, and R. Goldbach. 1995. Broad resistance to tospoviruses in transgenic tobacco plants expressing three tospoviral nucleoprotein gene sequences. *Mol. Plant-Microbe Interact.* 8:85-91.
140. Prins, M., R. de Oliveira Resende, C. Anker, A. van Schepen, P. de Haan, and R. Goldbach. 1996. Engineered RNA-mediated resistance to tomato spotted wilt virus is sequence specific. *Mol. Plant- Microbe Interact.* 9:416-418.
141. Prins, M., M. Kikkert, C. Ismayadi, W. de Graauw, P. de Haan, and R. Goldbach. 1997. Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants expressing NS(M) gene sequences. *Plant Mol. Biol.* 33:235-243.
142. Prüfer, D., J. Schmitz, E. Tacke, B. Kull, and W. Rohde. 1997. In vivo expression of a full-length cDNA copy of potato leafroll virus (PLRV) in protoplasts and transgenic plants. *Mol. Gen. Genet.* 253:609-614.
143. Quemada, H.D., D. Gonsalves, and J.L. Slightom. 1991. Expression of coat protein gene from cucumber mosaic virus strain C in tobacco: protection against infections by CMV strains transmitted mechanically or by aphids. *Phytopathology* 81:794-802.

144. Rao, K.V., K.S. Rathore, T.K. Hodges, X. Fu, E. Stoger, D. Sudhaker, S. Williams, P. Christou, M. Bharathi, D.P. Brown, K.S. Powell, J. Spence, A.M. Gatehouse and J.A. Gatehouse. 1998. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. *Plant J.* 15:469-477.
145. Ravelonandro, M., M. Monsion, R. Delbos, and J. Dunez. 1993. Variable resistance to plum pox virus and potato virus Y infection in transgenic *Nicotiana* plants expressing plum pox virus coat protein. *Plant Science* 91:157-169.
146. Reavy, B., M. Arif, S. Kashiwazaki, K.D. Webster, and H. Barker. 1995. Immunity to potato mop-top virus in *Nicotiana benthamiana* plants expressing the coat protein gene is effective against fungal inoculation of the virus. *Mol. Plant-Microbe Interact.* 8:286-291.
147. Regner, F., A. da Câmara Machado, M. Laimer da Câmara Machado, H. Steinkellner, D. Mattanovich, V. Hanzer, H. Weiss, and H. Katinger, H. 1992. Coat protein mediated resistance to plum pox virus in *Nicotiana clelandii* and *N. benthamiana*. *Plant Cell Rep.* 11:30-33.
148. Reimann-Philipp, U., and R. Beachy. 1993. Coat protein-mediated resistance in transgenic tobacco expressing the tobacco mosaic virus coat protein from tissue-specific promoters. *Mol. Plant-Microbe Interact.* 6: 323-330.
149. Rezaian, M.A., K.G.M. Skene, and J.G. Ellis. 1988. Anti-sense RNAs of cucumber mosaic virus in transgenic plants assessed for control of the virus. *Plant Mol. Biol.* 11:463-471.
150. Rogers, P., S. Whitby, and M. Dando. 1999. Biological warfare against crops. *Scientific American* June 70-75.
151. Roossinck, M.J., D. Sleat, and P. Palukaitis. 1992. Satellite RNAs of plant viruses: Structures and biological effects. *Microbiol. Rev.* 56: 265-279.
152. Rubino, L., and M. Russo. 1995. Characterization of resistance to cymbidium ringspot virus in transgenic plants expressing a full-length viral replicase gene. *Virology* 212:240-243.
153. Rubino, L., R. Lupo, and M. Russo. 1993. Resistance to cymbidium ringspot tomosvirus infection in transgenic *Nicotiana benthamiana* plants expressing a full-length viral replicase gene. *Mol. Plant-Microbe Interact.* 6:729-734.
154. Saito, Y., T. Komari, C. Masuta, Y. Hayashi, T. Kumashiro, and Y. Takanami. 1992. Cucumber mosaic virus-tolerant transgenic tomato plants expressing a satellite RNA. *Theor. Appl. Genet.* 83:679-683.
155. Sanders, P.R., B. Sammons, W. Kaniewski, L. Haley, J. Layton, B.J. LaVallee, X. Delannay, and N.E. Turner. 1992. Field resistance of transgenic tomatoes expressing the

tobacco mosaic virus or tomato mosaic virus coat protein genes. *Phytopathology* 82:683-690.

156. Schillberg, S., S. Zimmermann, A. Voss, and R. Fischer. 1999. Apoplastic and cytosolic expression of full-size antibodies and antibody fragments in *Nicotiana tabacum*. *Transgenic Res.* 8:255-263.
157. Scholthof, K.-B.G., H.B. Scholthof, and A.O. Jackson. 1993. Control of plant virus diseases by pathogen-derived resistance in transgenic plants. *Plant Physiol.* 102:7-12.
158. Seppänen, P., R. Puska, J. Honkanen, L.G. Tyulkina, O. Fedorkin, S.Y. Morozov, and J.G. Atabekov. 1997. Movement protein-derived resistance to triple gene block-containing plant viruses. *J. Gen. Virol.* 78:1241-1246.
159. Service, R.F. 1998. Seed-sterilizing 'Terminator Technology' sows discord. *Science* 282: 850-851.
160. Sijen, T., J. Wellink, J. Hendriks, J. Verver, and A. van Kammen. 1995. Replication of cowpea mosaic virus RNA1 or RNA2 is specifically blocked in transgenic *Nicotiana benthamiana* plants expressing the full-length replicase or movement protein genes. *Mol. Plant-Microbe Interact.* 8:340-347.
161. Sijmons, P.C., B.M. Dekker, B. Schrammeijer, T.C. Verwoerd, P.J. van den Elzen, and A. Hoekema. 1990. Production of correctly processed human serum albumin in transgenic plants. *Biotechnology* 8:217-221.
162. Smith, H.A., S.L. Swaney, T.D. Parks, E.A. Wernsman, and W.G. Dougherty. 1994. Transgenic plant virus resistance mediated by untranslatable sense RNAs: expression, regulation, and fate of nonessential RNAs. *Plant Cell* 6:1441-1453.
163. Smith, H.A., H. Powers, S. Swaney, C. Brown, and W.G. Dougherty. 1995. Transgenic potato virus Y resistance in potato: evidence for an RNA-mediated cellular response. *Phytopathology* 85:864-870.
164. Spillane, C., J. Verchot, T.A. Kavanagh, and D.C. Baulcombe. 1997. Concurrent suppression of virus replication and rescue of movement-defective virus in transgenic plants expressing the coat protein of potato virus X. *Virology* 236:76-84.
165. Stanley, J., T. Frischmuth, and S. Ellwood. 1990. Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants. *Proc. Natl. Acad. Sci. USA* 87: 6291-6295.
166. Stark, D.M., and R.N. Beachy. 1989. Protection against potyvirus infection in transgenic plants: evidence for broad spectrum resistance. *Bio/Technology* 7:1257-1262.

167. Stenger, D.C. 1994. Strain-specific mobilization and amplification of a transgenic defective- interfering DNA of the geminivirus beet curly top virus. *Virology* 203:397-402.
168. Suzuki, M., C. Masuta, Y. Takanami, and S. Kuwata. 1996. Resistance against cucumber mosaic virus in plants expressing the viral replicon. *FEBS Lett.* 379:26-30.
169. Swaney, S., H. Powers, J. Goodwin, L.S. Rosales, and W.G. Dougherty. 1995. RNA-mediated resistance with nonstructural genes from the tobacco etch virus genome. *Mol. Plant-Microbe Interact.* 8:1004-1011.
170. Tackaberry, E.S., A.K. Dudam, F. Prior, M. Tocchi, R. Sardana, I. Altosaar, and P.R. Ganz. 1999. Development of biopharmaceuticals in plant expression systems: cloning, expression and immunological reactivity of human cytomegalovirus glycoprotein B (UL55) in seeds of transgenic tobacco. *Vaccine* 17:3020-3029.
171. Tacke, E., F. Salamini, and W. Rohde. 1996. Genetic engineering of potato for broad-spectrum protection against virus infection. *Nature Biotech.* 14:1597-1601.
172. Takahashi, H., Z. Chen, H. Du, Y. Liu, and D.F. Klessig. 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *Plant J.* 11:993-1005.
173. Taliany, M.E., E.V. Ryabov, and D.J. Robinson. 1998. Two distinct mechanisms of transgenic resistance mediated by groundnut rosette virus satellite RNA sequences. *Mol. Plant-Microbe Interact.* 11:367-374.
174. Taschner, P.E.M., G. van Marle, F.Th. Brederode, N.E. Turner, and J.F. Bol. 1994. Plants transformed with a mutant alfalfa mosaic virus coat protein gene are resistant to the mutant but not to wild-type virus. *Virology* 203:269-276.
175. Tavladoraki, P., E. Benvenuto, S. Trinca, D. De Martinis, A. Cattaneo, and P. Galeffi. 1993. Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack. *Nature* 366:469-472.
176. Tenllado, F., I. Garcia-Luque, M.T. Serra, and J.R. Diaz-Ruiz. 1995. *Nicotiana benthamiana* plants transformed with the 54-kDa region of the pepper mild mottle tobamovirus replicase gene exhibit two types of resistance responses against viral infection. *Virology* 211:170-183.
177. Tenllado, F., I. Garcia-Luque, M.T. Serra, and J.R. Diaz-Ruiz. 1996. Resistance to pepper mild mottle tobamovirus conferred by the 54-kDa gene sequence in transgenic plants does not require expression of the wild-type 54-kDa protein. *Virology* 219:330-335.
178. Topfer, R., N. Martini, and J. Schell. 1995. Modification of plant lipid synthesis. *Science* 268: 681-686.

179. Tricoli, D.M., K.J. Carney, P.F. Russell, J.R. McMaster, D.W. Groffl, K.C. Hadden, P.T. Himmel, J.P. Hubbard, M.L. Boeshore, and H.D. Quemada. 1995. Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2, and zucchini yellow mosaic virus. *Bio/Technology* 13:1458-1465.
180. Truve, E., A. Aaspolu, J. Honkanen, R. Puska, M. Mehto, A. Hassi, T.H. Teeri, M. Kelve, P. Seppänen, and M. Saarima. 1993. Transgenic potato plants expressing mammalian 2'-5' oligoadenylate synthetase are protected from potato virus X infection under field conditions. *Bio/Technology* 11:1048-1052.
181. Turner, N.E., D.-J. Hwang, and M. Bonness. 1997. C-terminal deletion mutant of pokeweed antiviral protein inhibits viral infection but does not depurinate host ribosomes. *Proc. Natl. Acad. Sci. USA* 94:3866-3871.
182. Turner, N.E., K.M. O'Connell, R.S. Nelson, P.R. Sanders, R.N. Beachy, R.T. Fraley, and D.M. Shah. 1987. Expression of alfalfa mosaic virus coat protein gene confers cross-protection in transgenic tobacco and tomato plants. *EMBO J.* 6:1181-1188.
183. Vaira, A.M., L. Semeria, S. Crespi, V. Lisa, A. Allavena, and G.P. Accotto. 1995. Resistance to tospoviruses in *Nicotiana benthamiana* transformed with the N gene of tomato spotted wilt virus: correlation between transgene expression and protection in primary transformants. *Mol. Plant-Microbe Interact.* 8:66-73.
184. van der Vlugt, R.A.A., R.K. Ruiter, and R. Goldbach. 1992. Evidence for sense RNA-mediated protection to PVYN in tobacco plants transformed with the viral coat protein cistron. *Plant Mol. Biol.* 20:631-639.
185. van der Wilk, F., D.P.-L. Willink, M.J. Huisman, H. Huttinga, and R. Goldbach. 1991. Expression of the potato leafroll luteovirus coat protein gene in transgenic potato plants inhibits viral infection. *Plant Mol. Biol.* 17:431-439.
186. van Dun, C.M.P., and J.F. Bol. 1988. Transgenic tobacco plants accumulating tobacco rattle virus coat protein resist infection with tobacco rattle virus and pea early browning virus. *Virology* 167:649-652.
187. van Dun, C.M.P., J.F. Bol, and L. van Vloten-Doting. 1987. Expression of alfalfa mosaic virus and tobacco rattle virus coat protein genes in transgenic tobacco plants. *Virology* 159:299-305.
188. van Dun, C.M., B. Overduin, L. van Vloten-Doting, and J.F. Bol. 1988. Transgenic tobacco expressing tobacco streak virus or mutated alfalfa mosaic virus coat protein does not cross-protect against alfalfa mosaic virus infection. *Virology* 164:383-389.
189. Vardi, E., I. Sela, O. Edelbaum, O. Livneh, L. Kuznetsova, and Y. Stram. 1993. Plants transformed with a cistron of a potato virus Y protease (Nla) are resistant to virus infection. *Proc. Natl. Acad. Sci. USA* 90:7513-7517.

190. Voss, A., M. Niersbach, R. Hain, H.J. Hirsch, Y.C. Liao, F. Kreuzaler, and R. Fischer. 1995. Reduced virus infectivity in *N. tabacum* secreting a TMV-specific full-size antibody. *Mol. Breed.* 1:39-50.
191. Watanabe, Y., T. Ogawa, H. Takahashi, I. Ishida, Y. Takeuchi, M. Yamamoto, and Y. Okada. 1995. Resistance against multiple plant viruses in plants mediated by a double stranded-RNA specific ribonuclease. *FEBS Lett.* 372:165-168.
192. Whitham, S., S. McCormick, and B. Baker. 1996. The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. *Proc. Natl. Acad. Sci. USA* 93:8776-8781.
193. Whitham, S., S.P. Dinesh-Kumar, D. Choi, R. Hehl, C. Corr, and B. Baker. 1994. The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. *Cell* 78:1101-1115.
194. Wigdorovitz, A., D.M. Perez Filgueira, N. Robertson, C. Carrillo, A.M. Sadir, T.J. Morris, and M.V. Borca. 1999. Protection of mice against challenge with foot and mouth disease virus (FMDV) by immunization with foliar extracts from plants infected with recombinant tobacco mosaic virus expressing the FMDV structural protein VP1. *Virology* 264:85-91.
195. Wilson, T.M.A. 1993. Strategies to protect crop plants against viruses: pathogen-derived resistance blossoms. *Proc. Natl. Acad. Sci. USA* 90:3134-3141.
196. Wintermantel, W.M., N. Banerjee, J.C. Oliver, D.J. Paolillo, and M. Zaitlin. 1997. Cucumber mosaic virus is restricted from entering minor veins in transgenic tobacco exhibiting replicase-mediated resistance. *Virology* 231:248-257.
197. Wittner, A., L. Palkovics, and E. Balazs. 1998. *Nicotiana benthamiana* plants transformed with the plum pox virus helicase gene are resistant to virus infection. *Virus Res.* 53:97-103.
198. Wisniewski, L.A., P.A. Powell, R.S. Nelson, and R.N. Beachy. 1990. Local and systemic spread of tobacco mosaic virus in transgenic tobacco. *Plant Cell* 2:559-567.
199. Wolf, S., C.M. Deom, R.N. Beachy, and W.J. Lucas. 1989. Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. *Science* 246:377-379.
200. Yepes, L.M., M. Fuchs, J.L. Slightom, and D. Gonsalves. 1996. Sense and antisense coat protein gene constructs confer high levels of resistance to tomato ringspot nepovirus in transgenic *Nicotiana* species. *Phytopathology* 86:417-424.
201. Yie, Y., F. Zhao, S.Z. Zhao, Y.Z. Liu, Y.L. Liu, and P. Tien. 1992. High resistance to cucumber mosaic virus conferred by satellite RNA and coat protein in transgenic commercial tobacco cultivar G-140. *Mol. Plant-Microbe Interact.* 5:460-465.

202. Zaccomer, B., F. Cellier, J.-C. Boyer, A.-L. Haenni, and M. Tepfer. 1993. Transgenic plants that express genes including the 3' untranslated region of the turnip yellow mosaic virus (TYMV) genome are partially protected against TYMV infection. *Gene* 136:87-94.
203. Zaitlin, M., J.M. Anderson, K.L. Perry, L. Zhang, and P. Palukaitis. 1994. Specificity of replicase-mediated resistance to cucumber mosaic virus. *Virology* 201:200-205.
204. Zeitlin, L., S.S. Olmsted, T.R. Moench, M.S. Co, B.J. Martinell, V.M. Paradkar, D.R. Russell, C. Queen, R.A. Cone, and K.J. Whaley. 1998. A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes. *Nat. Biotechnol.* 16:1361-1364.

Appendix A

Production of a metric tonne of a biological toxin in potatoes

In the evaluation of the production of eucaryotic biologicals in plants, potatoes are used as a model plant expression system. The protein content in potatoes can reach 25% of dry matter and the dry matter content of potato is typically between 20% and 30%. Assuming the heterologous protein comprises 10% of the total protein, it should be possible to produce approximately 8 kg of a biological toxin per metric tonne of potatoes. Potatoes are perhaps the most economical plant production system, since price is approximately \$100 CDN per ton. Other plant systems are available for the production of products that require extremely high levels of purity, but costs increase accordingly.

The DNA sequences of many biological toxins have been published, and the ease of cloning genes, using the polymerase chain reaction, would provide the necessary cDNA for the toxin gene. Once the gene is available, it would take approximately 3 months to clone into an appropriate plasmid (*Agrobacterium* or plant virus-based vector), using conventional cloning techniques. The recombinant plant plasmid would then be transfected into the appropriate plant cells using *Agrobacterium* transfection or microprojectile bombardment. Regeneration in plant tissue culture and recovery of stable transgenic plants would take another three months. Potatoes plants can then be grown and expanded as for other potato seed stock. A crude protein preparation can be made by a simple pressing of the potato and collection of the liquid. The level of expertise required to perform the procedure would be graduate or postdoctoral training in molecular biology and plant tissue culture skills. Although the post-translational processing in a plant may differ from other eukaryotic expression systems, specific activities have not been reduced to date even in complex molecules.

In concert with Defence Research Establishment Suffield, Agriculture Canada will produce a transgenic potato plant expressing a recombinant humanized antibody as a model biological. Expression of a recombinant antibody will be evaluated for level of expression, biological function, glycosylation, the formation of disulphide bonds and the ability to be secreted into the extracellular space.

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The objective of this report is to bring to light the current state of plant biotechnology, and to evaluate implications that may impact potential BW threats. The mechanism of xenogenic gene expression in plants is reviewed, along with examples of vaccines and antibodies which have been produced in plants. The economics and virtually unlimited quantities of material that can be produced, are the principle advantages of this eucaryotic expression system. The potential to use plants as a production system for biological toxins is a concern that warrants more detailed analysis.

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